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(71) Applicant *(for all designated States except US)*: **BIOMEDISINSK INNOVASJON AS** [NO/NO]; Gaustadalleen 21, N-0349 Oslo (NO).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **SAATCIOGLU, Fahri** [TR/NO]; Gaupefaret 23, N-0773 Oslo (NO).

(74) Agent: **ONSAGERS AS**; P.O.Box 6963 St. Olavs plass, N-0130 Oslo (NO).

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(54) Title: STAMP2 NUCLEIC ACID MOLECULES, POLYPEPTIDES, AND DIAGNOSTIC AND THERAPEUTIC METHODS

(57) Abstract: The invention provides novel STAMP2 nucleic acid molecules, polypeptides, and antibodies for use in methods of diagnosing, treating, and preventing diseases and conditions of the prostate and testis, such as cancer.

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STAMP2 NUCLEIC ACID MOLECULES, POLYPEPTIDES, AND DIAGNOSTIC AND THERAPEUTIC METHODS

Background of The Invention

Genitourinary disorders are often difficult to diagnose and treat effectively because the patients often present non-specifically. Two causes of genitourinary disorders are disorders of the prostate gland and the testis.

The prostate is a variable sized gland located in the male pelvis, and is made up of several different cell types, including epithelial cells and stromal cells. Prostate-associated disorders include prostate cancer, benign prostatic hyperplasia, and prostatitis. The male hormone testosterone and other androgen related hormones have major roles in the growth and function of the prostate. The testis is also subject to many defects, including developmental anomalies, inflammation, and cancer.

In men, prostate cancer is the most commonly diagnosed cancer and the second leading cause of cancer mortality following skin cancer. Androgens have a critical role in the development and maintenance of the male reproductive system and, in the initial stages of prostate cancer, the cancer is dependent on androgens for growth. This dependence is the basis for androgen ablation therapy. In most cases, however, prostate cancer progresses to an androgen-independent phenotype for which there is no effective therapy available at present.

Despite the recognition of the role of androgens in prostate biology and pathophysiology for more than 60 years, the molecular mechanisms that are responsible for the effects of androgens are largely unknown. Recent studies have begun to identify androgen-regulated and prostate-enriched genes and the proteins that they encode. These studies have also helped to define the molecular details of

androgen action in the prostate and in prostate carcinogenesis. The first such gene discovered was Kallikrein 3 (*KLK3*), also called prostate-specific antigen (PSA), the detection of which is currently used as a diagnostic tool and also as a marker for the progression of prostate cancer, albeit with significant limitations due to variations in the patient population. More recently, several additional prostate-enriched genes were identified including prostate-specific membrane antigen (PSMA), prostate carcinoma tumor antigen 1 (PCTA-1), NKX3.1, prostate stem cell antigen (PSCA), DD3, and PCGEM1. Other androgen regulated genes that are primarily expressed in the prostate include KLK2, KLK4, NKX3.1, and PCGEM1.

It would be beneficial to provide reagents, such as androgen-regulated genes and probes for detection of such genes that are useful for the diagnosis and therapy and identification of therapeutics for disorders associated with the prostate and the testis, as well as other tissues.

Summary of the Invention

We have discovered that STAMP2, a six-transmembrane protein is expressed at very high levels in prostate tissue and localizes primarily to the Golgi complex, the plasma membrane, and the early endosomes, I believe this indicates that STAMP2 may be involved in the secretory and endocytic pathways. Our findings also indicate that STAMP2 may be involved in the induction of cell proliferation and growth. We have also discovered that *STAMP2* is an androgen responsive gene in androgen receptor positive prostate cancer cells, but not in androgen receptor negative cells. Similarly, STAMP2 is an androgen responsive polypeptide in androgen receptor positive prostate cancer cells, but not in androgen receptor negative cells. Furthermore, STAMP2 expression is increased in a subset of prostate cancers compared with matched normal prostate epithelial

cells microdissected from the same radical prostatectomy specimens. The invention provides, in general, nucleic acids, polypeptides, antibodies, and modulatory compounds for use in methods of diagnosing, treating, and preventing diseases and conditions of the prostate and testis, such as cancer.

Accordingly, in a first aspect the invention features a method of diagnosing a subject as having, or having a propensity to develop, a disorder of the prostate or testis that includes measuring the level of a STAMP2 polypeptide in a sample from the subject. In preferred embodiments, an agent (e.g., an antibody) that specifically binds to STAMP2 is used to measure the level of STAMP2 polypeptide. In additional preferred embodiments, the measuring of the STAMP2 polypeptide levels can be performed using an immunological assay such as an ELISA and an antibody that specifically binds STAMP2. In preferred embodiments, the method also includes comparing the level of STAMP2 polypeptide in a subject sample to a reference sample or level of STAMP2 polypeptide.

In another aspect, the invention features a method of diagnosing a subject as having, or having a propensity to develop, a disorder of the prostate or testis that includes measuring the level of a STAMP2 polynucleotide or a polynucleotide encoding a STAMP2 polypeptide or fragments thereof (collectively referred to as STAMP2 nucleic acids) nucleic acid in a sample from the subject. Any of the STAMP2 nucleic acid molecules described below (or a portion thereof sufficient for specificity) can be used as a probe for STAMP2 nucleic acids in the diagnostic methods of the invention. In preferred embodiments, the measuring of the STAMP2 polynucleotide levels can be performed using a nucleic acid probe that specifically hybridizes to STAMP2 nucleic acids. In preferred embodiments, the method also includes comparing the level of a *STAMP2* nucleic acid from a subject sample to a reference sample or level of a *STAMP2* nucleic acid.

In preferred embodiments of the diagnostic aspects of the invention, the reference sample is a prior sample obtained from the same subject. In additional preferred embodiments, the reference standard or level is a level or number derived from such a sample. The reference standard or level can also be a value derived from a normal subject that is matched to the sample subject by at least one of the following criteria: age, family history or prostate or testis disorders, and weight. In additional preferred embodiments, the reference sample is a normal control taken from a subject that does not have a disorder of the testis or prostate or a purified protein at known normal concentrations. If the reference sample or level is a normal reference, an increase (e.g., at least 10%, 25%, 50%, 75% or more) in the level of STAMP2 polypeptide or nucleic acid is a diagnostic indicator of a disorder of the prostate or testis.

In preferred embodiments of any of the above methods of the invention, the measuring of the levels of STAMP2 polypeptide or polynucleotide is done on two or more occasions and an alteration (e.g., an increase of at least 10%, 25%, 50%, 75% or more) in the levels between measurements is a diagnostic indicator of a disorder, or propensity to develop a disorder, of the prostate or testis.

In another aspect, the invention features a method of diagnosing a subject as having, or having a propensity to develop, a disorder of the prostate or testis, said method comprising determining the nucleic acid sequence of a *STAMP2* gene in a sample from a subject and comparing it to a reference sequence. In this method, an alteration or polymorphism in the subject's nucleic acid sequence that changes the expression level or biological activity of the *STAMP2* gene product in the subject diagnoses the subject with a disorder of the prostate, or a propensity to develop a disorder of the prostate or testis.

In preferred embodiments of any of the above aspects, the sample used for the diagnostic methods is blood, serum, urine, semen, cerebrospinal fluid, saliva,

or a cell or tissue (e.g., a cell or tissue biopsy sample derived from a prostate or testis). In additional preferred embodiments, the subject is a mammal (e.g., human, a cow, a horse, a sheep, a pig, a goat, a dog, and a cat).

The diagnostic methods of the invention can be used for screening or diagnosis of a disorder of the prostate or testis, or for monitoring the effectiveness of therapy.

In yet another aspect, the invention features a kit for the diagnosis of a disorder of the prostate or testis in a subject, which includes a component for detecting a STAMP2 polypeptide or any fragment thereof. In preferred embodiments, the component includes the use of an immunological assay, an enzymatic assay, and a colorimetric assay. In additional preferred embodiments, the kit includes a STAMP2 binding agent (e.g., an antibody) that specifically binds a STAMP2 polypeptide.

In another related aspect, the invention features a kit for the analysis of a *STAMP2* nucleic acid molecule, which includes a *STAMP2* nucleic acid molecule probe at least 80% identical, preferably at least 86%, most preferably at least 90% 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to *STAMP2* cDNA or a fragment thereof, wherein the probe specifically hybridizes under high stringecy conditions to the sequence set forth in SEQ ID NO: 1 or the complementary sequences thereof. In preferred embodiments, the probe includes a the polynucleotide that is at least 80%, preferably 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to nucleotides 107 to 167 or to nucleotides 1306 to 1360 of SEQ ID NO: 1. In additional preferred embodiments, the probe includes a polynucleotide that is at least 80%, preferably 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a nucleic acid molecule that encodes STAMP2 (SEQ ID NO: 2) or amino acids 1 to 20, amino acids 70 to 82, amino acids 87 to 97, amino acids

330 to 347, amino acids 400 to 428, or amino acids 445 to 459 of STAMP2 (SEQ ID NO: 2).

In another aspect, the invention features a substantially pure polynucleotide comprising a sequence at least 80%, preferably at least 86%, and most preferably at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to *STAMP2* cDNA (SEQ ID NO: 1) or a fragment thereof. In preferred embodiments, the polynucleotide consists of the *STAMP2* cDNA sequence. In preferred embodiments, the polynucleotide has STAMP2 biological activity.

The invention also features a substantially pure polynucleotide comprising a nucleic acid sequence encoding a STAMP2 polypeptide having an amino acid sequence at least 80%, preferably at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the STAMP2 amino acid sequence set forth in SEQ ID NO: 2. In preferred embodiments, the polynucleotide consists of the nucleic acid sequence encoding the STAMP2 polypeptide sequence set forth in SEQ ID NO: 2.

In another aspects the invention features a substantially pure polynucleotide comprising a sequence at least 80%, preferably at least 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to nucleotides 107 to 167 of *STAMP2* cDNA (SEQ ID NO: 1). The invention also features a substantially pure polynucleotide comprising a sequence at least 80%, preferably at least 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to nucleotides 1306 to 1360 of *STAMP2* cDNA (SEQ ID NO: 1). The invention also features a substantially pure polynucleotide comprising a sequence at least 80%, preferably at least 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of amino acids 1 to 20, amino acids 70 to 82, amino acids 87 to 97, amino acids 330 to 347, amino acids 400 to 428, or amino acids 445 to 459 of STAMP2 (SEQ ID NO: 2).

In another aspect, the invention features a substantially pure polynucleotide comprising a sequence that hybridizes at high stringency to the STAMP2 nucleic

acid sequence of SEQ ID NO: 1, or a fragment thereof. In a related aspect, the invention features a substantially pure polynucleotide comprising a sequence that hybridizes at high stringency to a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 2, or a fragment thereof. Preferred fragments include polynucleotides encoding amino acids 1 to 20, amino acids 70 to 82, amino acids 87 to 97, amino acids 330 to 347, amino acids 400 to 428, or amino acids 445 to 459 of STAMP2 (SEQ ID NO: 2).

Any of the polynucleotides described above can be used as nucleic acid probes or primers to detect STAMP2 nucleic acids, for example, in the diagnostic methods of the invention.

In another aspect, the invention features a substantially pure polypeptide comprising a sequence at least 80%, preferably at least 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to STAMP2 (SEQ ID NO: 2). In additional aspects, the polypeptide includes a sequence that is at least 80%, preferably at least 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acids 1 to 20, amino acids 70 to 82, amino acids 87 to 97, amino acids 330 to 347, amino acids 400 to 428, or amino acids 445 to 459 of STAMP2 (SEQ ID NO: 2).

In additional aspects, the invention features an antibody that specifically binds to a polypeptide that includes the sequence of STAMP2 (SEQ ID NO: 2) or amino acids 1 to 20, amino acids 70 to 82, amino acids 87 to 97, amino acids 330 to 347, amino acids 400 to 428, or amino acids 445 to 459 of STAMP2 (SEQ ID NO: 2).

In another aspect, the invention features a method of diagnosing a subject as having, or having a propensity to develop, a disorder of the prostate or testis that includes measuring the level of a STAMP2 polypeptide in a sample from the subject. In additional preferred embodiments, the measuring of the STAMP2 polypeptide levels can be performed using an immunological assay such as an

ELISA and an antibody that specifically binds STAMP2. In preferred embodiments, the method also includes comparing the level of STAMP2 polypeptide in a subject sample to a reference sample or level of STAMP2 polypeptide.

In another aspect, the invention features a method of diagnosing a subject as having, or having a propensity to develop, a disorder of the prostate or testis that includes measuring the level of a STAMP2 polynucleotide or a polynucleotide encoding a STAMP2 polypeptide or fragments thereof (collectively referred to as STAMP2 nucleic acids) nucleic acid in a sample from the subject. Any of the STAMP2 nucleic acid molecules described herein can be used as a probe in these diagnostic methods. In preferred embodiments, the measuring of the STAMP2 polynucleotide levels can be performed using a nucleic acid probe that specifically hybridizes to STAMP2 nucleic acids. In preferred embodiments, the method also includes comparing the level of a *STAMP2* nucleic acid from a subject sample to a reference sample or level of a *STAMP2* nucleic acid.

In preferred embodiments of the diagnostic aspects of the invention, the reference sample is a prior sample obtained from the same subject. In additional preferred embodiments, the reference standard or level is a level or number derived from such a sample. The reference standard or level can also be a value derived from a normal subject that is matched to the sample subject by at least one of the following criteria: age, family history or prostate or testis disorders, and weight. In additional preferred embodiments, the reference sample is a normal control taken from a subject that does not have a disorder of the testis or prostate or a purified protein at known normal concentrations. If the reference sample or level is a normal reference, an increase (e.g., at least 10%, 25%, 50%, 75% or more) in the level of STAMP2 polypeptide or nucleic acid is a diagnostic indicator of a disorder of the prostate or testis.

In preferred embodiments of any of the above methods of the invention, the measuring of the levels of STAMP2 polypeptide or polynucleotide is done on two or more occasions and an alteration (e.g., an increase of at least 10%, 25%, 50%, 75% or more) in the levels between measurements is a diagnostic indicator of a disorder, or propensity to develop a disorder, of the prostate or testis.

In another aspect, the invention features a method of diagnosing a subject as having, or having a propensity to develop, a disorder of the prostate or testis, said method comprising determining the nucleic acid sequence of a *STAMP2* gene in a sample from a subject and comparing it to a reference sequence. In this method, an alteration or polymorphism in the subject's nucleic acid sequence that changes the expression level or biological activity of the *STAMP2* gene product in the subject diagnoses the subject with a disorder of the prostate, or a propensity to develop a disorder of the prostate or testis.

In preferred embodiments of any of the above aspects, the sample used for the diagnostic methods is blood, serum, urine, semen, cerebrospinal fluid, saliva, or a cell or tissue (e.g., a cell or tissue biopsy sample derived from a prostate or testis).

In yet another aspect, the invention features a kit for the diagnosis of a disorder of the prostate or testis in a subject, which includes a component for detecting a STAMP2 polypeptide or any fragment thereof. In preferred embodiments, the component includes the use of an immunological assay, an enzymatic assay, and a colorimetric assay. In additional preferred embodiments, the kit includes an antibody that specifically binds a STAMP2 polypeptide.

In another related aspect, the invention features a kit for the analysis of a a *STAMP2* nucleic acid molecule, which includes a *STAMP2* nucleic acid molecule probe at least 80% identical, preferably at least 86%, most preferably at least 90% 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to *STAMP2* cDNA or a

fragment thereof, wherein the probe specifically hybridizes under high stringecy conditions to the sequence set forth in SEQ ID NO: 1 or the complementary sequences thereof. In preferred embodiments, the probe includes a the polynucleotide that is at least 80%, preferably 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to nucleotides 107 to 167 or to nucleotides 1306 to 1360 of SEQ ID NO: 1. In additional preferred embodiments, the probe includes a polynucleotide that is at least 80%, preferably 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a nucleic acid molecule that encodes STAMP2 (SEQ ID NO: 2) or amino acids 1 to 20, amino acids 70 to 82, amino acids 87 to 97, amino acids 330 to 347, amino acids 400 to 428, or amino acids 445 to 459 of STAMP2 (SEQ ID NO: 2).

In another aspect, the invention features a method of treating a subject having a disorder of the prostate or testis that includes administering a compound capable of decreasing the biological activity or the expression level of STAMP2 gene or protein. In preferred embodiments, the compound is a nucleobase oligomer that is at least 90%, 91%, 92%, 93%, 94%, 96%, 96%, 97%, 98%, 99%, or 100% complementary to at least a portion of a *STAMP2* nucleic acid sequence. The nucleobase oligomer can be an antisense nucleobase oligomer, having at least one strand that is at least 90%, 91%, 92%, 93%, 94%, 96%, 96%, 97%, 98%, 99%, or 100% complementary to at least 8 to 30 consecutive nucleotides of the desired nucleic acid sequence, where the antisense nucleobase oligomer can reduce or inhibit the expression of endogenous STAMP2 gene or protein. The antisense nucleobase oligomer can also contain at least 40, 60, 85, 120, or more consecutive nucleotides that are complementary to STAMP2 mRNA or DNA, and may be as long as the full-length mRNA or gene.

The nucleobase oligomer can also be a double stranded RNA (dsRNA), preferably a small interfering RNA (siRNA), having at least one strand that is

preferably at least 90%, 91%, 92%, 93%, 94%, 96%, 96%, 97%, 98%, 99%, or 100% complementary to 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more consecutive nucleotides of the desired STAMP2 nucleic acid sequence, where the dsRNA can reduce or inhibit the expression of endogenous STAMP2 gene or protein. The dsRNA can also contain at least 40, 60, 85, 120, or more consecutive nucleotides that are complementary to STAMP2 mRNA or DNA, and may be as long as the full-length mRNA or gene.

Any of the nucleobase oligomers used in the therapeutic methods of the invention are administered in an amount and for a time sufficient to reduce or inhibit the expression of a STAMP2 gene or polypeptide.

In additional preferred embodiments of this aspect, the compound is an antibody or antigen-binding fragment, preferably a monoclonal antibody, that specifically binds STAMP2. In preferred embodiments, the antibody or antigen-binding fragment thereof is a human or humanized antibody. In additional preferred embodiments, the antibody specifically binds amino acids 1 to 20, amino acids 70 to 82, amino acids 87 to 97, amino acids 330 to 347, amino acids 400 to 428, or amino acids 445 to 459 of STAMP2 (SEQ ID NO: 2).

The methods of the present invention can be used to diagnose or treat the disorders described herein in any mammal, for example, humans, domestic pets, or livestock. Where a non-human mammal is treated or diagnosed, the STAMP2 polypeptide, nucleic acid molecule, or antibody employed is preferably specific for that species.

In preferred embodiments, the methods of the invention also include the use of any combination of nucleic acid molecules of the invention to treat a subject having a disorder of the prostate or testis. In a preferred embodiment, the subject is a mammal, preferably a human and the disorder is prostate cancer. In other preferred embodiments of any of the above aspects, the therapeutic nucleic acid

molecules are stably expressed in a cell (e.g., a mammalian, human, or neoplastic cell). In preferred embodiments of any of the above aspects, the human cell is *in vivo*.

In another aspect, the invention features a vector comprising a nucleic acid molecule positioned for expression, where the nucleic acid molecule encodes a nucleic acid molecule having at least one strand that is substantially complementary to at least a portion of the sequence of a STAMP2 nucleic acid molecule. In preferred embodiments, the nucleic acid molecule is a STAMP2 polynucleotide as described above. In additional preferred embodiments, the nucleic acid molecule is a double stranded nucleic acid molecule, an siRNA molecule or an antisense molecule. In additional preferred embodiments the nucleic acid molecule is an siRNA molecule that has 100% nucleic acid sequence identity to at least 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides of SEQ ID NO: 1 or a nucleic acid molecule encoding SEQ ID NO: 2. In yet additional preferred embodiments, the nucleic acid molecule is an antisense nucleobase oligomer molecule that is complementary to at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 nucleotides of SEQ ID NO: 1 or a nucleic acid molecule encoding SEQ ID NO: 2.

In another aspect, the invention features a method of identifying a compound that ameliorates a disorder of the prostate or testis that includes contacting a cell that expresses a *STAMP2* nucleic acid molecule with a candidate compound, and comparing the level of expression of the *STAMP2* nucleic acid molecule in the cell contacted by the candidate compound with the level of expression in a control cell not contacted by the candidate compound. An alteration (e.g., a decrease of at least 10%, 25%, 50%, 75% or more) in expression of the *STAMP2* nucleic acid molecule identifies the candidate compound as a compound that ameliorates a disorder of the prostate or testis.

In another aspect, the invention features a method of identifying a compound that ameliorates a disorder of the prostate or testis that includes contacting a cell that expresses a STAMP2 polypeptide with a candidate compound and comparing the level of expression of the STAMP2 polypeptide in the cell contacted by the candidate compound with the level of polypeptide expression in a control cell not contacted by the candidate compound. An alteration (e.g., a decrease of at least 10%, 25%, 50%, 75% or more) in the expression of the STAMP2 polypeptide identifies the candidate compound as a compound that ameliorates a disorder of the prostate or testis. In preferred embodiments, the alteration in expression is assayed using an immunological assay, an enzymatic assay, or an immunoassay.

In another aspect, the invention features a method of identifying a compound that ameliorates a disorder of the prostate or testis, the method comprising contacting a cell that expresses a STAMP2 polypeptide with a candidate compound, and comparing the biological activity of the STAMP 2 polypeptide in the cell contacted by the candidate compound with the level of biological activity in a control cell not contacted by the candidate compound, wherein an alteration in the biological activity of the STAMP2 polypeptide identifies the candidate compound as a compound that ameliorates a disorder of the prostate or testis.

Any of the nucleic acids, polypeptides and antibodies described above can be used in therapeutic or diagnostic methods of the invention described below.

By "alteration" is meant a change (increase or decrease) in the expression levels or biological activity of a gene or polypeptide as detected by standard art known methods such as those described above. As used herein, an increase or decrease includes a 10% change, preferably a 25% change, more preferably a

50%, 75%, 95% or greater change in expression levels or biological activity of STAMP2.

By "antisense nucleobase oligomer" is meant a nucleobase oligomer, regardless of length, that is complementary to the coding strand or mRNA of a *STAMP2* gene. The antisense nucleobase oligomer can also be targeted to the translational start and stop sites. The antisense nucleobase oligomer can contain at least 8, 10, 20, or 30 nucleotides in length. Other preferred lengths include 40, 60, 85, 120, or more consecutive nucleotides that are complementary to mRNA or DNA encoding a STAMP2 protein, and may be as long as the full-length mRNA or gene. Preferably the antisense nucleobase oligomer comprises from about 8 to 30 nucleotides.

By "disorder of the prostate or testis" is meant a disturbance of function and/or structure of the prostate or testis in a living organism, resulting from an external source, a genetic predisposition, a physical or chemical trauma, or a combination of any of the above. Such disorders include the proliferation of prostate or testicular cells. Exemplary disorders include prostate cancer, benign prostatic hyperplasia, acute prostatitis, testicular cancer, and developmental defects of the prostate or testis (such as cryptorchidism or undescended testis, and retractile, ascending, or vanished testis). Additional examples of disorders of the prostate or testis can be found in Campbell's Urology, Seventh Edition, W.B. Saunders Company, Philadelphia (1998).

By "expression" is meant the detection of a gene or polypeptide by standard art known methods. For example, polypeptide expression is often detected by immunological assays such as western blotting, DNA expression is often detected by Southern blotting or polymerase chain reaction (PCR), and RNA expression is often detected by northern blotting, PCR, or RNAse protection assays.

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or more nucleotides or amino acids.

By "homologous" is meant any gene or protein sequence that bears at least 30% homology, more preferably 40%, 50%, 60%, 70%, 80%, and most preferably 90%, 95%, 96%, 97%, 98%, 99% or 100% or more homology to a known gene or protein sequence over the length of the comparison sequence. A "homologous" protein can also have at least one biological activity of the comparison protein. For polypeptides, the length of comparison sequences will generally be at least 10 amino acids, preferably at least 15 amino acids, more preferably at least 20 amino acids, and most preferably at least 25 amino acids or more up to the length of the polypeptide. For nucleic acids, the length of comparison sequences will generally be at least 15 nucleotides, preferably at least 30 nucleotides, more preferably at least 40 nucleotides, and most preferably at least 50 nucleotides up to the length of the nucleic acid. "Homology" can also refer to a substantial similarity between an epitope used to generate antibodies and the protein or fragment thereof to which the antibodies are directed. In this case, homology refers to a similarity sufficient to elicit the production of antibodies that can specifically recognize the protein at issue.

By "hybridize" is meant pair to form a double-stranded molecule between complementary polynucleotide sequences, or portions thereof, under various conditions of stringency. (See, e.g., Wahl and Berger (1987) *Methods Enzymol.* 152:399; Kimmel, *Methods Enzymol.* 152:507, 1987.) In one embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and

1% SDS. In a more preferred embodiment, hybridization will occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 μg/ml denatured salmon sperm DNA (ssDNA). In another embodiment, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 42%, 45%, or 50% formamide, and 200 μg/ml ssDNA. Generally, by "high stringency conditions" is meant conditions that allow hybridization comparable with the hybridization that occurs using a DNA probe of at least 500 nucleotides in length, in a buffer containing 0.5 M NaHPO4, pH 7.2, 7% SDS, 1 mM EDTA, and 1% BSA (fraction V), at a temperature of 65° C., or a buffer containing 48% formamide, 4.8X SSC, 0.2 M Tris-Cl, pH 7.6, 1X Denhardt's solution, 10% dextran sulfate, and 0.1% SDS, at a temperature of 42° C, 65° C, or 68° C. (These are typical conditions for high stringency northern or Southern hybridizations.) High stringency hybridization is also relied upon for the success of numerous techniques routinely performed by molecular biologists, such as high stringency PCR, DNA sequencing, single strand conformational polymorphism analysis, and in situ hybridization. In contrast to northern and Southern hybridizations, these techniques are usually performed with relatively short probes (e.g., usually 16 nucleotides or longer for PCR or sequencing, and 40 nucleotides or longer for in situ hybridization). For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C.

Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25°C, more preferably of at least about 42°C, and most preferably of at least about 68°C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art.

Hybridization techniques and high stringency conditions are well known to those skilled in the art and are described, for example, in Benton and Davis (*Science* 196:180, 1977); Grunstein and Hogness (*Proc. Natl. Acad. Sci.*, USA 72:3961, 1975); Ausubel et al. (*Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001); Berger and Kimmel (*Guide to Molecular Cloning Techniques*, 1987, Academic Press, New York); and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York.

The term "identity" is used herein to describe the relationship of the sequence of a particular nucleic acid molecule or polypeptide to the sequence of a reference molecule of the same type. For example, if a polypeptide or nucleic acid molecule has the same amino acid or nucleotide residue at a given position, compared to a reference molecule to which it is aligned, there is said to be "identity" at that position.

The level of sequence identity of a nucleic acid molecule or a polypeptide to a reference molecule is typically measured using sequence analysis software with the default parameters specified therein, such as the introduction of gaps to achieve an optimal alignment. Methods to determine identity are available in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package (Devereux et al., *Nucleic Acids Research* 12(1): 387, 1984), BLASTP, BLASTN, and FASTA (Altschul et al., *J. Mol. Biol.* 215: 403 (1990). The well-known Smith-Waterman algorithm may also be used to determine identity. The BLAST and BLAST2 programs are publicly available from NCBI and other sources (*BLAST Manual*, Altschul, et al., NCBI NLM NIH Bethesda, MD 20894). Searches can be performed in URLs such as http://www.ncbi.nlm.nih.gov/BLAST or http://www.ncbi.nlm.nih.gov/gorf/bl2.html (Tatusova et al., *FEMS Microbiol.* Lett. 174:247-250, 1999). These software programs match similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

A nucleic acid molecule or polypeptide is said to be "substantially identical" to a reference molecule if it exhibits, over its entire length, at least 50%,

60%, or 70%, preferably at least 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% to the sequence of the reference molecule. For polypeptides, the length of comparison sequences is at least 10 amino acids, preferably at least 15 amino acids or at least 20 amino acids, more preferably at least 25 amino acids, and most preferably, the full-length polypeptide. For nucleic acid molecules, the length of comparison sequences is at least 20 nucleotides, preferably at least 30 nucleotides, more preferably at least 40 nucleotides or at least 50 nucleotides, and most preferably, the full-length nucleic acid molecule. Alternatively, or additionally, two nucleic acid sequences are "substantially identical" if they hybridize under high stringency conditions.

In the context of amino acid sequence comparisons, the term "identity" is used to express the percentage of amino acid residues at the same relative positions that are the same. Also in this context, the term "homology" is used to express the percentage of amino acid residues at the same relative positions that are either identical or are similar, using the conserved amino acid criteria of BLAST analysis, as is generally understood in the art.

By "isolated nucleic acid molecule," "isolated polynucleotide," "substantially pure nucleic acid molecule," or "substantially pure and isolated nucleic acid molecule" is meant a nucleic acid molecule (for example, DNA or RNA) that is free of the genes that, in the naturally occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the nucleic acid. The term includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also

includes a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

By "nucleic acid," "nucleobase oligomers," or "polynucleotide" is meant a chain of at least eight nucleobases joined together by linkage groups. Included in this definition are natural and non-natural oligonucleotides, both modified and unmodified, as well as oligonucleotide mimetics such as Protein Nucleic Acids, locked nucleic acids, and arabinonucleic acids.

By "pharmaceutically acceptable carrier" is meant a carrier that is physiologically acceptable to the treated mammal while retaining the therapeutic properties of the compound with which it is administered. One exemplary pharmaceutically acceptable carrier is physiological saline solution. Other physiologically acceptable carriers and their formulations are known to one skilled in the art and described, for example, in Remington's Pharmaceutical Sciences, (20^{th} edition), ed. A. Gennaro, 2000, Lippincott, Williams & Wilkins, Philadelphia, PA.

By "polypeptide," "protein," or "polypeptide fragment" is meant a chain of two or more amino acids, regardless of any post-translational modification (e.g., glycosylation or phosphorylation), constituting all or part of a naturally or non-naturally occurring polypeptide. By "post-translational modification" is meant any change to a polypeptide or polypeptide fragment during or after synthesis. Post-translational modifications can be produced naturally (such as during synthesis within a cell) or generated artificially (such as by recombinant or chemical means). A protein can be made up of one or more polypeptides.

By "probe" is meant a single-stranded DNA or RNA molecule of defined sequence that can base pair to a second DNA or RNA molecule that contains a complementary sequence ("target"). The stability of the resulting hybrid depends upon the extent of the base pairing that occurs. This stability is affected by

parameters such as the degree of complementarity between the probe and target molecule, and the degree of stringency of the hybridization conditions. The degree of hybridization stringency is affected by parameters such as the temperature, salt concentration, and concentration of organic molecules, such as formamide, and is determined by methods that are well known to those skilled in the art. Probes that specifically bind to or hybridize to *STAMP2* nucleic acid molecules, preferably, have greater than 45% sequence identity, more preferably at least 55-75% sequence identity, still more preferably at least 75-85% sequence identity, yet more preferably at least 85-95% sequence identity, and most preferably 96%, 97%, 98%, 99% or 100% sequence identity to the nucleic acid sequences encoding the amino acid sequences described herein. Probes can be detectably-labeled, either radioactively or non-radioactively, by methods that are well-known to those skilled in the art. Probes can be used for methods involving nucleic acid hybridization, such as nucleic acid sequencing, nucleic acid amplification by the polymerase chain reaction, single stranded conformational polymorphism (SSCP) analysis, restriction fragment polymorphism (RFLP) analysis, Southern hybridization, northern hybridization, *in situ* hybridization, electrophoretic mobility shift assay (EMSA), and other methods that are well known to those skilled in the art. Probes can be 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or more nucleotides in length. Preferred probe fragments are described under the definition for "STAMP2 polynucleotide" or "STAMP2 polypeptide", below.

A molecule, e.g., an oligonucleotide probe or primer, a gene or fragment thereof, a cDNA molecule, a polypeptide, or an antibody, can be said to be "detectably-labeled" if it is marked in such a way that its presence can be directly identified in a sample. Methods for detectably-labeling molecules are well known in the art and include, without limitation, radioactive labeling (e.g., with an

isotope, such as ^{32}P or ^{35}S) and nonradioactive labeling (e.g., with a fluorescent label, such as fluorescein, or by generating a construct containing green fluorescent protein (GFP)).

By "RNA interference (RNAi)" is meant the administration of a nucleic acid molecule (e.g., antisense, shRNA, siRNA, dsRNA), regardless of length, that inhibits the expression of a STAMP2 nucleic acid molecule, or any fragment thereof. Typically, the administered nucleic acid molecule contains one strand that is complementary to the coding strand of a STAMP2 nucleic acid molecule, or any fragment thereof. RNAi is a form of post-transcriptional gene silencing initiated by the introduction of double-stranded RNA (dsRNA) or antisense RNA. Preferably, RNAi is capable of decreasing the expression of a STAMP2 nucleic acid molecule, polypeptide or any fragment thereof in a cell by at least 10%, 20%, 30%, or 40%, more preferably by at least 50%, 60%, or 70%, and most preferably by at least 75%, 80%, 90%, 95% or more. The double stranded RNA or antisense RNA is at least 8, 10, 20, or 30 nucleotides in length. Other preferred lengths include 40, 60, 85, 120, or more consecutive nucleotides that are complementary to a STAMP2 nucleic acid molecule, or any fragment thereof. The double stranded nucleic acid may contain a modified backbone, for example, phosphorothioate, phosphorodithioate, or other modified backbones known in the art, or may contain non-natural internucleoside linkages. In one preferred embodiment, short 19, 20, 21, 22, 23, 24, or 25 nucleotide double stranded RNAs are used to down regulate the expression or biological activity a STAMP2 nucleic acid molecule or polypeptide, or any fragment thereof and may be used, for example, as therapeutics to treat a disorder of the prostate or testis. Such RNAs are effective at down-regulating gene expression in mammalian tissue culture cell lines (Elbashir et al., *Nature* 411:494-498, 2001, hereby incorporated by

reference). The further therapeutic effectiveness of this approach in mammals was demonstrated *in vivo* by McCaffrey et al. (*Nature* 418:38-39. 2002).

By "small interfering RNA" or "siRNA" is meant an isolated RNA molecule, preferably greater than 10 nucleotides in length, more preferably greater than 15 nucleotides in length, and most preferably 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length that is used to identify a target gene or mRNA to be degraded. A range of 19-25 nucleotides is the most preferred size for siRNAs. siRNAs can also include short hairpin RNAs (shRNA) in which both strands of an siRNA duplex are included within a single RNA molecule. Double-stranded siRNAs generally consist of a sense and anti-sense strand. Single-stranded siRNAs generally consist of only the anti-sense strand that is complementary to the target gene. siRNA includes any form of RNA, preferably dsRNA (proteolytically cleaved products of larger dsRNA, partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA) as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution, and/or alteration of one or more nucleotides. Such alterations can include the addition of non-nucleotide material, such as to the end(s) of the 21 to 23 nucleotide RNA or internally (at one or more nucleotides of the RNA). In a preferred embodiment, the RNA molecule contains a 3'hydroxyl group. Nucleotides in the RNA molecules of the present invention can also comprise non-standard nucleotides, including non-naturally occurring nucleotides or deoxyribonucleotides. The double-stranded oligonucleotide may contain a modified backbone, for example, phosphorothioate, phosphorodithioate, or other modified backbones known in the art, or may contain non-natural internucleoside linkages. Additional modifications of siRNAs (e.g., 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, "universal base" nucleotides, 5-C-methyl nucleotides, one or more phosphorothioate internucleotide linkages, and inverted

deoxyabasic residue incoporation) can be found in the published U.S. application publication number 20040019001 (see Summary of the Invention section). Collectively, all such altered RNAs are referred to as modified siRNAs.

siRNAs of the present invention need only be sufficiently similar to natural RNA such that it has the ability to mediate RNAi. As used herein "mediate RNAi" refers to the ability to distinguish or identify which RNAs are to be degraded. Preferably, RNAi is capable of decreasing the expression of a STAMP2 nucleic acid molecule, polypeptide, or any fragment thereof in a cell by at least 10%, 20%, 30%, or 40%, more preferably by at least 50%, 60%, or 70%, and most preferably by at least 75%, 80%, 90%, 95% or more. In one preferred embodiment, short 21, 22, 23, 24, or 25 nucleotide double stranded RNAs are used to down regulate STAMP2 polypeptide expression (Elbashir et al., *Nature* 411:494-498, 2001).

By "shRNA" is meant an RNA comprising a duplex region complementary to an mRNA. For example, a short hairpin RNA (shRNA) may comprise a duplex region containing nucleotides, where the duplex is between 19 and 29 bases in length, and the strands are separated by a single-stranded 3, 4, 5, 6, 7, 8, 9, or 10 base linker region. Optimally, the linker region is 6 bases in length.

By "sample" is meant blood, serum, semen, urine, stool, saliva, cerebrospinal fluid, tissue biopsy, cells (e.g., prostate or testis tissue or cells), or other specimen obtained from a subject. The sample is analyzed to detect expression levels or a mutation in a gene encoding a polypeptide that is substantially identical to *STAMP2*, to detect expression levels or biological activity of a polypeptide substantially identical to STAMP2, or expression levels of a gene encoding a polypeptide that is substantially identical to STAMP2, as for example, an indication of the progression of cancer, by methods that are known in the art or described herein. For example, methods such as sequencing, single-strand conformational polymorphism (SSCP) analysis, or restriction fragment

length polymorphism (RFLP) analysis of PCR products derived from a patient sample may be used to detect a mutation in a gene encoding a polypeptide that is substantially identical to STAMP2; ELISA may be used to measure levels of polypeptide that is substantially identical to STAMP2; and PCR may be used to measure the level of nucleic acids encoding a polypeptide that is substantially identical to STAMP2.

A "reference sample" can include a sample, a level or a value representing a known level of STAMP2 associated with a disease or non-disease state. For example, a normal reference sample can be a sample taken from a different tissue or cell of the same subject, a different sample from the same subject known to be unaffected by any disorder of the prostate or testis, a sample previously taken from the same subject, or a sample taken from a different subject known to be unaffected by a disorder of the prostate or testis. A normal reference sample can also include a purified sample of STAMP2 gene or polypeptide at a known normal level or a standard curve using purified STAMP2 gene or polypeptide. The reference sample can also be a value representing a known normal level of STAMP2. A disease reference sample can be a sample or a level or value taken from a subject known to have a disorder of the prostate or testis.

By "specifically binds" is meant a compound or antibody which recognizes and binds a STAMP2 polypeptide but that does not substantially recognize and bind other molecules indistinguishable from STAMP2, in a sample, for example, a biological sample, which naturally includes a polypeptide of the invention. Desirably, the compound or antibody that specifically binds will bind a STAMP2 polypeptide to a greater extent than any other polypeptide, but the compound or antibody may detect minor additional polypeptides of a size or mobility that have detectably different characteristics (e.g., size, carbohydrate modifications, phosphorylation, peptidase sensitivity, and gel electrophoresis mobility) than

STAMP2. Specifically binds can also refer to a nucleic acid probe which recognizes and binds or hybridizes to a *STAMP2* nucleic acid molecule but does not substantially recognize and bind other nucleic acid molecules in a sample. Again, the probe may detect minor additional nucleic acids of a size or mobility that is detectably different than *STAMP2* nucleic acids.

By "STAMP2 biological activity" is meant exhibiting properties that contribute to extracellular trafficking pathways, secretory or endocytic pathways, or cell signaling pathways, and stimulation of cell proliferation or cell growth pathways. Any of the above activities can be assayed using techniques known in the art or described herein.

By "*STAMP2* polynucleotide" is meant a polynucleotide having a sequence that is at least 80%, preferably at least 86%, most preferably at least 90% 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence set forth in SEQ ID NO: 1. Included in this definition are fragments of STAMP2 polynucleotides that are at least 20 nucleotides in length, preferably 40 nucleotides, more preferably 50 nucleotides, and most preferably at least 60, 100, 200, 300, 400, 500, or more nucleotides in length. Preferred fragments include the nucleotide sequences that encode the amino-terminal half of the STAMP2 polypeptide up to the sequences encoding the six transmembrane domains (i.e., amino acids 1 to 225), nucleotide sequences encoding the carboxy-terminal half of the polypeptide including the six transmembrane domains (i.e., amino acids 225 to 459); or nucleotide sequences that encode, for example, amino acids 1 to 20, amino acids 70 to 82, amino acids 87 to 97, amino acids 330 to 347, amino acids 400 to 428, or amino acids 445 to 459, or any portion thereof. One preferred fragment includes nucleotides 1306-1360.

By "STAMP2 polypeptide" is meant a polypeptide having a sequence that is at least 80%, preferably at least 86%, most preferably at least 90% 90%, 95%,

96%, 97%, 98%, 99% or 100% identical to the sequence set forth in SEQ ID NO: 2. Included in this definition are fragments of STAMP2 which are at least 10 amino acids in length, preferably 15 amino acids in length, and most preferably at least 25 amino acids in length. Preferred fragments of STAMP2 are the amino-terminal half of the polypeptide up to the sequences encoding the six transmembrane domains (i.e., amino acids 1 to 225), the carboxy-terminal half of the polypeptide including the six transmembrane domains (i.e., amino acids 225 to 459), or fragments including, for example, amino acids 1 to 20, amino acids 70 to 82, amino acids 87 to 97, amino acids 330 to 347, amino acids 400 to 428, or amino acids 445 to 459 or any portion thereof. All numbering is based on the numbering of the polynucleotide and polypeptide sequences set forth in SEQ ID NOS: 1 and 2.

By "STAMP2 subcellular localization" is meant localization of STAMP2 protein to the Golgi, the plasma membrane, endoplasmic reticulum, or the early endosome.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline.

By "substantially pure polypeptide" or "substantially pure and isolated polypeptide" is meant a polypeptide (or a fragment thereof) that has been separated from components that naturally accompany it. Typically, the polypeptide is substantially pure when it is at least 60%, by weight, free from the proteins and naturally occurring organic molecules with which it is naturally associated. Preferably, the polypeptide is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, pure. A substantially pure polypeptide may be obtained by standard techniques, for example, by extraction from a natural source (e.g., prostate or testis tissue or cell lines), by expression of a recombinant nucleic acid encoding a prostate-specific or a testis-specific

polypeptide, or by chemically synthesizing the polypeptide. Purity can be measured by any appropriate method, e.g., by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

A protein or polypeptide is substantially free of naturally associated components when it is separated from those contaminants that accompany it in its natural state. Thus, a protein that is chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. Accordingly, substantially pure polypeptides not only include those derived from eukaryotic organisms but also those chemically synthesized or sythesized in *E. coli* or other prokaryotes.

By "therapeutic amount" is meant an amount that when administered to a patient suffering from a disorder of the prostate or testis is sufficient to cause a qualitative or quantitative reduction in the symptoms of the disorder of the prostate or testis. A "therapeutic amount" can also mean an amount that when administered to a patient suffering from a disorder of the prostate or testis is sufficient to cause a reduction in the expression levels of STAMP2 gene or protein as measured by the assays described herein.

By "treating" is meant administering a compound or a pharmaceutical composition for prophylactic and/or therapeutic purposes. To "treat disease" or use for "therapeutic treatment" refers to administering treatment to a subject already suffering from a disease to improve the subject's condition. To "prevent disease" refers to prophylactic treatment of a subject who is not yet ill, but who is susceptible to, or otherwise at risk of, developing a particular disease. Thus, in the claims and embodiments, treating is the administration to a mammal either for therapeutic or prophylactic purposes.

Other features and advantages of the invention will be apparent from the detailed description of the invention, the drawings, and the claims.

Brief Description of The Drawings

Figure 1A shows the gene structure of the STAMP2 gene. Boxes denote exons. The sizes of the introns and exons are shown. The location of the predicted start and stop codons are indicated with black and grey arrows, respectively.

Figure 1B shows the relative location of *STAMP1, STAMP2*, and *STEAP* genes on Chr7q. The telemoric (Tel) and centromeric (Centr) ends of the chromosome are indicated. The BAC clones encompassing the genes, as well as the distance between the genes, are indicated. The arrows indicate direction of transcription 5' to 3'.

Figure 2 shows the cDNA sequence (SEQ ID NO: 1) and amino acid sequence (SEQ ID NO: 2) of STAMP2 and the locations of the predicted transmembrane domains are underlined. The N-terminal domain that has sequence similarity to dinucleotide binding motifs, oxidoreductases, or pyrroline 5-carboxylate reductase, is shown in bold.

Figure 3 shows a sequence alignment of STAMP2 with TIARP (GenBank accession number NP473439), STAMP1 (GenBank accession number AAG32149), TSAP6 (GenBank accession number AAH42150), pHyde (GenBank accession number AAK00361.1), and STEAP (GenBank accession number AF186249) obtained by Clustal and GenDoc programs. Completely conserved residues are shaded in black; residues that are conserved in four or five of the sequences are shaded light and dark gray, respectively.

Figure 4A is an autoradiogram showing the results of a multiple tissue Northern blot (Clontech) probed with *STAMP2* or *G3PDH* cDNA. The lanes

represent: 1.Heart, 2. Brain, 3. Placenta, 4. Lung, 5. Liver, 6. Skeletal Muscle, 7. Kidney, 8. Pancreas, 9. Spleen, 10. Thymus, 11. Prostate, 12. Testis, 13. Ovary, 14. Small Intestine, 15. Colon, 16. Peripheral blood leukocyte. The location of the full-length 4.0 kb mRNA is indicated by arrows to the left of the figure. The stronger hybridization that is observed with *G3PDH* in the heart and skeletal muscle samples is due to its higher expression in these tissues.

Figure 4B is an autoradiogram showing the results of a Northern blot analysis of total RNA from LNCaP, PC3, or DU145 cells either left untreated or treated with the synthetic androgen R1881 (10^{-8} M) for 24 hours as indicated, and probed with *STAMP2*. *STAMP2* expression was also assessed in a number of prostate cancer cell lines not expressing the androgen receptor (PC-3, DU-145, CA-HPV10, PZ-HPV7, YPEN-1), in myotubes (C2), lung cancer cells (NCI-H661), breast cancer cells (MCF7, MCF7-LCC1, MCF7-LCC2, MB435), or cervical carcinoma cells (HeLa), as indicated. The same blot was also probed with a *STAMP1* cDNA probe.

Figure 4C is an autoradiogram showing the results of a Northern blot analysis of total RNA isolated from LNCaP cells either left untreated or treated with R1881 (10^{-8} M) for the indicated time points.

Figure 5A is a series of images showing the intracellular localization of GFP-STAMP2. COS-1 cells were transiently transfected with GFP-STAMP2 and then fixed and processed for confocal microscopy as described below. A series of confocal sections were collected through a single cell along the z-axis at 100 nm intervals, one of which is shown in low and high exposure, to the left and the right, respectively. Areas of intracellular GFP-STAMP2 localization are indicated with arrows: Golgi; plasma membrane, PM; vesiculotubular structures, VTS; and endoplasmic reticulum (ER).

Figure 5B is a series of images showing the colocalization of GFP-STAMP2 with Golgi and TGN markers. COS-1 cells transfected with GFP-STAMP2 were fixed and labeled with antibodies against well characterized Golgi and TGN markers: β-coat protein (β -COP), giantin, and TGN46. Green GFP-STAMP2 fluorescence and red (Texas Red-labeled secondary antisera) β -COP, giantin, and TGN46 fluorescence were detected by confocal laser microscopy. For each row, the image in the right column shows the overlay of the prior two images with yellow/orange staining indicating the regions of overlap. The areas marked by a rectangle are enlarged and shown as insets.

Figures 6 is a series of images showing time-lapse microscopy of GFP-STAMP2 trafficking in living cells. A COS-1 cell transiently expressing GFP-STAMP2 was observed by live-cell confocal microscopy at 37°C. Eight consecutive images were taken at 3 second intervals. The movement of a particular vesicular structure from cytoplasm to plasma membrane is shown (white arrows) within the region of the cell that is magnified (white square). Note that the results shown are representative of multiple time-lapse analyses and the changes in the images are not due to movement from the plain of focus.

Figure 7 is a series of images showing the colocalization of GFP-STAMP2 with the early endosomal marker EEA1 as detected by indirect immunofluorescence microscopy. COS-1 cells transfected with GFP-STAMP2 were fixed and labeled with antibodies against the well-characterized early endosomal marker EEA1. Green GFP-STAMP2 fluorescence and red (Texas Red-labeled secondary antiserum) EEA1 fluorescence were detected by confocal laser microscopy. The right-hand panel shows the overlay images with yellow/orange staining indicating the regions of overlap. The areas marked by white rectangles are enlarged and shown as insets.

Figure 8 is a graph showing the results of quantitative RT-PCR analysis of *STAMP2* expression in microdissected, matched normal vs neoplastic prostate glands. Sections were obtained from radical prostatectomy specimens that were snap-frozen upon resection, and subjected to Laser Capture Microdissection (LCM). A pathologist procured normal and cancer glands from the same tissue (total of 26 matched pairs, from different patients). Total RNA was isolated, and used for cDNA synthesis and quantitative RT-PCR with *STAMP2*-specific primers. The graph shows representative results obtained from two independent experiments.

Figure 9 is an autoradiogram showing the results of a western blot analysis of whole cell extracts isolated from LNCaP cells either left untreated or treated with R1881 (10^{-8} M) for the indicated times. Extracts were separated by SDS-PAGE, transferred to a PVDF membrane and probed using an antiserum raised against a peptide corresponding to amino acids 445-459 of STAMP2.

Figure 10A is a photograph of two representative dishes of cell showing colony formation in the DU145 cells expressing vector alone and DU145 expressing STAMP2 cDNA. Figure 10B is a graph showing the percent total area of the colonies for the DU145 cells expressing vector alone and DU145 cells expressing STAMP2. The results shown are from experiments done in triplicate repeated at least three times.

Figure 11 is a graph showing the ratio of the number of COS7 cells expressing STAMP2 to COS7 cells expressing GFP over time. The results shown are from experiments done in triplicate repeated at least 3 times.

Figure 12 is a graph showing the ratio of the number of DU145 cells expressing STAMP2 to the number of DU145 cells expressing GFP over time. The results shown are from experiments done in triplicate repeated at least 3 times.

Detailed Description of the Invention

The basic biology of the normal prostate and testis, as well as pathological conditions relating to the prostate and testis, is still poorly understood. Androgens are known to have a critical role in both normal and pathological conditions relating to the prostate. We have identified, cloned, and characterized STAMP2, a six transmembrane protein that is highly enriched in prostate tissues and cell lines. STAMP2 localizes to the Golgi, endosomes and plasma membrane suggesting that STAMP2 may play a role in endocytic/secretory trafficking pathways. We have also discovered that STAMP2 may play a role in cell proliferation and that STAMP2 is an androgen responsive gene which is specifically upregulated in androgen receptor positive prostate cancer cells.

The invention provides STAMP2 specific polypeptides, nucleic acid molecules, and antibodies that are useful for diagnostic and therapeutic methods for the diagnosis and treatment of disorders of the prostate, such as cancer.

STAMP2 nucleic acid molecules and polypeptides

We have discovered that STAMP2 is a six transmembrane protein that is androgen-regulated in prostate cancer cells expressing the androgen receptor, and is localized to the plasma membrane, the Golgi, and endosomes. We have also discovered that although STAMP2 shares homology with a distinct mouse protein, TIARP, and a rat protein pHyde, there are regions of STAMP2 that are unique and may be used to specifically target STAMP2 nucleic acids and polypeptides. Fragments of STAMP2 that encompass these regions are particularly useful in the diagnostic and therapeutic methods of the invention described below.

Preferred regions of STAMP2 include the amino-terminal half of the protein which does not include the six transmembrane domains (i.e., amino acids 1 to 225), the carboxy-terminal half (i.e., amino acids 225 to 459), which includes

the six transmembrane domains, amino acids 1-20, amino acids 70-82, amino acids 87-97, amino acids 330-347, amino acids 400-428, and amino acids 445 to 459. Polypeptides can include only these regions or can include these regions and additional sequences. STAMP2-containing polypeptides (e.g., chimeric fusion proteins) can also be used to raise antibodies specific for various regions of STAMP2 polypeptides.

Preferred polynucleotides include STAMP2 polynucleotides, or fragments thereof, or any nucleic acids that encode any of the above polypeptide fragments. One example of a preferred nucleic acid sequence includes nucleic acids 1306-1360. The preferred nucleic acid sequences can be used to design antisense nucleic acids or siRNAs for downregulation of STAMP2 gene expression. In one example, an antisense oligonucleotide to STAMP2 includes the nucleic acid sequence that is complementary to nucleotides 1306 to 1360 of *STAMP2*. Full length STAMP2 nucleic acids or fragments thereof can also be used as probes for the detection of STAMP2. Such probes are useful for example in the diagnostic methods described herein.

Methods for generating such fragments are well known in the art (see, for example, Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY, 1998), using the nucleotide sequences provided herein. For example, a STAMP2 polynucleotide fragment can be generated by PCR amplifying a desired STAMP2 nucleic acid molecule fragment using oligonucleotide primers designed based upon the STAMP2 nucleic acid sequences. Preferably, the oligonucleotide primers include unique restriction enzyme sites that facilitate insertion of the amplified fragment into the cloning site of an expression vector (e.g., a mammalian expression vector). This vector can then be introduced into a cell (e.g., a mammalian cell) using any of the various techniques known in the art such as those described herein, resulting in the

production of a STAMP2 polypeptide fragment in the cell containing the expression vector.

Synthesis of STAMP2 Proteins, Polypeptides, and Polypeptide Fragments

Those skilled in the art of molecular biology will understand that a wide variety of expression systems can be used to produce recombinant STAMP2 proteins. The precise host cell used is not critical to the invention. The STAMP2 proteins can be produced in a prokaryotic host (e.g., *E. coli*) or in a eukaryotic host (e.g., *S. cerevisiae*, insect cells such as Sf9 cells, or mammalian cells such as COS, NIH 3T3, CHO, or HeLa cells). These cells are commercially available from, for example, the American Type Culture Collection, Rockville, MD (see also Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY, 1998). The method of transformation and the choice of expression vehicle (e.g., expression vector) will depend on the host system selected. Transformation and transfection methods are described, (e.g., in Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY, 1998, and expression vehicles can be chosen from those provided, e.g. in Pouwels *et al.*, *Cloning Vectors: A Laboratory Manual*, 1985, Supp. 1987).

The characteristics of *STAMP2* nucleic acid molecules are analyzed by introducing such genes into various cell types or using *in vitro* extracellular systems. The function of STAMP2 proteins produced in such cells or systems are then examined under different physiological conditions. Also, cell lines can be produced that overexpress the STAMP2 gene product, allowing purification of STAMP2 proteins for biochemical characterization, large-scale production, antibody production, and patient therapy.

The polypeptides of the invention may be produced *in vivo* or *in vitro*, and may be chemically and/or enzymatically modified. The polypeptides can be

isolated from prostate tissue or prostate cancer cells that may or may not be in a hormone dependent state. Alternatively, and especially where larger amounts (i.e., >10mg) are desirable, recombinant production (e.g., in a bacterial, yeast, insect cell, or mammalian cell system) may advantageously be employed to generate significant quantities of STAMP2 polypeptides.

Recombinant production not only offers a more economical strategy to produce the polypeptides of the invention, but also allows specific modification in the amino acid sequence and composition to tailor particular biochemical, catalytic and physical properties. For example, where increased solubility of STAMP2 is desirable, one or more hydrophobic amino acids may be replaced with hydrophilic amino acids. Alternatively, where reduced or increased biological activity is required, one or more amino acids may be replaced or eliminated.

In still another example, the polypeptides of the invention can be synthesized as fusion proteins including, for example, fusions with enzymatically active partners (e.g., for dye formation or substrate conversion) and fluorescent partners such as GFP, EGFP, and BFP and variants thereof.

With respect to chemical and enzymatic modifications of contemplated polypeptides, many modifications are appropriate, including addition of mono-, and bifunctional linkers, coupling with protein- and non-protein macromolecules, and glycosylation. For example, mono- and bifunctional linkers are especially advantageous where polypeptides are immobilized to a solid support, or covalently coupled to a molecule that enhances immunogenicity of contemplated polypeptides (e.g., KLH, or BSA conjugation). Alternatively, the polypeptides may be coupled to antibodies or antibody fragments to allow rapid retrieval of the polypeptide from a mixture of molecules. Further couplings include covalent and non-covalent coupling of polypeptides with molecules that prolong the serum half-

life and/or reduce immunogenicity such as cyclodextranes and polyethylene glycols.

Diagnostic Methods

STAMP2 nucleic acid molecules, polypeptides, and antibodies are used in methods to diagnose or monitor a variety of diseases and conditions, including those involving mutations in, or inappropriate expression of STAMP2 polypeptides or nucleic acids.

The diagnostic methods of the invention are used, for example, with subjects that have a disorder of the prostate or testis, for example, prostate or testicular cancer, in an effort to determine its etiology, and thus, to facilitate selection of an appropriate course of treatment. The diagnostic methods are also used with subjects that have not yet developed a disorder of the prostate or testis, but who may be at risk of developing such a disease (e.g., a patient with a family history of disorders of the prostate or testis), or with subjects that are at an early stage of developing such a disease. Many disorders of the prostate or testis occur during development, and thus, the diagnostic methods of the invention are also carried out on a fetus or embryo during development. Also, the diagnostic methods of the invention are used in prenatal genetic screening, for example, to identify parents who may be carriers of a mutation in a *STAMP2* gene.

Disorders of the prostate or tesis can be detected using the diagnostic methods of the invention include those characterized by, for example, (i) abnormal (e.g., increased levels or inappropriate localization) expression of STAMP2 polypeptides or nucleic acid molecules, (ii) mutations in a STAMP2 gene that result in the production of such polypeptides, (iii) mutations in a STAMP2 gene or polypeptide that result in production of abnormal amounts of STAMP2

nucleotides or polypeptides, and (iv) alterations in a STAMP2 gene or polypeptide that affect the biological activity or subcellular localization of STAMP2.

Levels of *STAMP2* nucleic acid expression in a subject sample are determined by using any of a number of standard techniques that are well known in the art. In one embodiment, a subject having a disorder of the prostate or testis, or a propensity to develop such conditions, will show an increase (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more) in the level of *STAMP2* nucleic acid or a nucleic acid encoding a STAMP2 polypeptide or fragments thereof (collectively referred to as STAMP2 nucleic acids). Preferred fragments include nucleic acids encoding amino acids 1 to 20, amino acids 70 to 82, amino acids 87 to 97, amino acids 330 to 347, amino acids 400 to 428, or amino acids 445 to 459 of STAMP2 (SEQ ID NO: 2).

Methods for detecting such alterations in nucleic acid levels are standard in the art and are described in Ausubel et al., *supra*. Desirably, STAMP2 expression in a biological sample (e.g., a blood, prostate or testis tissue or cell sample, urine or semen) from a patient is monitored by standard northern blot analysis or by quantitative RT-PCR (see, e.g., Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY, 1998; *PCR Technology: Principles and Applications for DNA Amplification*, H.A. Ehrlich, Ed., Stockton Press, NY; Yap *et al. Nucl. Acids. Res.*19:4294, 1991). In another embodiment, nucleic acid probes that are capable of detecting a nucleic acid molecule encoding a polypeptide of the invention, including genomic sequences, or closely related molecules, may be used to hybridize to a *STAMP2* nucleic acid sequence from a subject. Preferred nucleic acid probes are specific for STAMP2 nucleic acids or fragments thereof and hybridize to STAMP2 nucleic acid molecules to a greater extent than any other nucleic acid molecule in the sample. Probes that specifically bind to or hybridize to *STAMP2* nucleic acid molecules, preferably, have greater

than 45% sequence identity, more preferably at least 55-75% sequence identity, still more preferably at least 75-85% sequence identity, yet more preferably at least 85-95% sequence identity, and most preferably 96%, 97%, 98%, 99% or 100% sequence identity to the nucleic acid sequences encoding the amino acid sequences described herein.

The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), determine whether the probe hybridizes to a naturally occurring sequence, allelic variants, or other related sequences. Hybridization techniques can be used for detection, prognosis, diagnosis, or monitoring of disorders of the prostate or testis. Hybridization techniques can also be used to identify mutations indicative of a disorder of the prostate or testis or a propensity to develop such a disorder or may be used to monitor expression levels of a STAMP2 nucleic acid (for example, by Northern analysis, Ausubel et al., *supra*).

In situ hybridization of RNA can be used to detect the expression of *STAMP2* genes. RNA *in situ* hybridization techniques rely upon the hybridization of a specifically labeled nucleic acid probe to the cellular RNA in individual cells or tissues. Therefore, RNA *in situ* hybridization is a powerful approach for studying tissue- and temporal-specific gene expression. In this method, oligonucleotides, cloned DNA fragments, or antisense RNA transcripts of cloned DNA fragments corresponding to unique portions of *STAMP2* genes are used to detect specific mRNA species, e.g., in the tissues of animals, such as mice, at various developmental stages, or to monitor tumor progression. Other gene expression detection techniques are known to those of skill in the art and can be employed for detection of *STAMP2* gene expression.

A mutant STAMP2 gene or a polymorphism can also be identified using these sources as test samples, for example, by mismatch detection techniques. Preferably, the DNA sample is subjected to PCR amplification prior to analysis. A biological sample obtained from a patient can be analyzed for one or more mutations in *STAMP2* nucleic acid molecules using a mismatch detection approach. Generally, this approach involves PCR amplification of nucleic acid molecules from a patient sample, followed by identification of a mutation (i.e., a mismatch) by detection of altered hybridization, aberrant electrophoretic gel migration, binding, or cleavage mediated by mismatch binding proteins, or by direct nucleic acid molecule sequencing. Any of these techniques can be used to facilitate detection of mutant prostate-specific or testis-specific genes, and each is well known in the art. Examples of these techniques are described, for example, by Orita et al. (*Proc. Natl. Acad. Sci. USA* 86:2766-2770, 1989) and Sheffield et al. (*Proc. Natl. Acad. Sci. USA* 86:232-236, 1989).

Mismatch detection assays also provide an opportunity to diagnose a STAMP2-mediated predisposition to a disease before the onset of symptoms. For example, a patient heterozygous for a *STAMP2* mutation that suppresses normal prostate-specific or testis-specific biological activity or expression may show no clinical symptoms of a *STAMP2* gene-related disease, and yet possess a higher than normal probability of developing a prostate or testicular disease. Given such a diagnosis, patients can take precautions to minimize their exposure to adverse environmental factors and to carefully monitor their medical condition (for example, through frequent physical examinations).

In yet another diagnostic approach of the invention, levels of STAMP2 polypeptide are measured or monitored in a biological sample. Desirably, an immunoassay is used to measure STAMP2 polypeptide levels. Anti-STAMP2 polypeptide polyclonal or monoclonal antibodies, such as those described herein,

can be used in any standard immunoassay format (e.g., ELISA, Western blot, or RIA; see, e.g., Ausubel *et al.*, *supra*) to measure STAMP2 polypeptide levels. These levels are desirably compared to a normal reference for STAMP2 levels. For example, an increase (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more) in STAMP2 polypeptide production may be indicative of a disorder of the prostate or testis or a predisposition to such a condition.

There are a variety of assay formats known to those of ordinary skill in the art for measuring the level of a STAMP2 polypeptide in a sample, including, without limitation, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, 2-dimensional gel electrophoresis, competitive and non-competitive assay systems using techniques such as Western blots, immunocytochemistry, immunohistochemistry, immunoassays, e.g., radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitation reactions, gel diffusion precipitation reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays and protein A immunoassays (See also, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory). In general, a disorder of the prostate or testis in a subject may be determined by (a) contacting a biological sample obtained from a subject with an agent (e.g., an antibody) that specifically binds STAMP2; (b) detecting in the sample a level of STAMP2 polypeptide that binds to the agent; and (c) comparing the level of STAMP2 polypeptide with a reference value. Reference values may be determined by methods known in the art, such as by establishing ranges of expression that give degrees of confidence in distinguishing a sample indicative of a disorder of the prostate or testis from a normal sample.

The diagnostic assays described above can be carried out using any biological sample (for example, a blood, serum, urine, semen, saliva, prostate or testis tissue or cell sample, or amniotic fluid) in which a STAMP2 polypeptide or nucleic acid molecule is normally expressed. In one example, the measurement of STAMP2 nucleic acids or polypeptides described herein preferably occurs on at least two different occasions and an alteration (e.g., increase) in the levels over time is used as an indicator of a disorder of the prostate or testis, or a propensity to develop such conditions. In another example, the measurement of any of the STAMP2 nucleic acids or polypeptides described herein is compared to a reference. If the reference is a normal reference, an increase is an indicator of a disorder of the prostate or testis, or a propensity to develop such conditions.

In a preferred example, a combined diagnostic method can be employed that includes an evaluation of STAMP2 protein expression (for example, by immunological techniques or the protein truncation test (Hogerrorst *et al.*, *Nature Genetics* 10:208-212, 1995), and a nucleic acid molecule-based detection technique designed to identify more subtle *STAMP2* mutations (for example, point mutations). As described above, a number of mismatch detection assays are available to those skilled in the art, and any preferred technique can be used. Mutations in STAMP2 genes can be detected that either result in loss or gain of STAMP2 polypeptide or nucleic acid molecule expression or biological activity.

STAMP2 polypeptides or nucleic acid molecules can be used to correlate the course of prostate cancer to a marker other than PSA, to monitor the course of an anticancer therapy, or to detect a neoplastic cell in a system. For example, a predetermined level of *STAMP2* RNA is correlated with the presence of a neoplastic cell, for example, from a biopsy. The total RNA is extracted from the biopsy specimen, and a real time quantitative RT-PCR employing individual reactions with primer pairs specific to *STAMP2* sequences is performed in parallel

with a biopsy specimen known to be free of cancer cells. Biopsy specimens are determined to have a cancer cell, where the detected *STAMP2* mRNA level is at least 5 times higher than in the control specimen. In still other aspects of contemplated methods, the polypeptide level need not necessarily be limited to at least 5 times more than the control specimen in order to establish that the tissue has a cancer cell. For example, where the concentration of the polypeptide is hormone dependent, amounts between 3 to 8 fold and more may be appropriate. In contrast, where the concentration of cancer cells in the biopsy specimen is relatively low, levels of less than 5-fold, including 1.5 to 4.9-fold and less are contemplated.

In alternative aspects of the inventive subject matter, the method of detecting a neoplastic cell need not be limited to biopsy tissues from prostate or testis tissue, but may employ various alternative tissues, including lymphoma tumor cells, and various solid tumor cells, so long as such tumor cells overproduce *STAMP2* mRNA. Likewise, the system need not be restricted to a subject, but may also include cell and tissue cultures grown *in vitro*. For example, tumor cell and tissue grown *in vitro* may advantageously be utilized to investigate drug action on such cells, and STAMP2 polynucleotides and polypeptides may conveniently be employed as a tumor marker. Alternatively, body fluids (e.g., serum, saliva, semen, blood, and urine) that may or may not contain tumor cells are also contemplated a suitable substrate for the method presented herein, so long as they contain a detectable level of STAMP2 mRNA.

The methods of detecting STAMP2 nucleotides and polypeptides can also be used to monitor specific treatment regimens *in vivo*. For example, since STAMP2 is an androgen-regulated gene, androgen withdrawal therapy can be monitored by detection of expression levels of STAMP2 nucleotides and polypeptides. In this example, a sample from a subject known to have a disorder

of the prostate or testis, is tested for STAMP2 polynucleotide or polypeptide expression levels. During the course of androgen withdrawal therapy, samples from the subject are taken at specific intervals and monitored for STAMP2 polynucleotide or polypeptide expression levels. Androgen withdrawal therapy should result in a decrease in the levels of STAMP2 polynucleotide or polypeptide expression levels and this decrease can be used to monitor the effectiveness of the androgen withdrawal therapy.

The detection process may include fluorescence detection, colorimetric detection, luminescence detection, scintigraphy, autoradiography, immunological assays, and formation of a dye. For example, for microscopic analysis of biopsy specimens, luciferase labeled probes are particularly advantageous in conjunction with a luminescence substrate (e.g., luciferin). Luminescence quantification may then be performed utilizing a CCD-camera and image analysis system. Similarly, radioactivity may be detected via autoradiographic or scintigraphic procedures on a tissue section, in a fluid or on a solid support. Where the probe is a natural or synthetic ligand of a STAMP2 polypeptide, the ligand may include molecules with a chemical modification that increases the affinity to the polypeptide and/or induce irreversible binding to the polypeptide. For example, transition state analogs or suicide inhibitors for a particular reaction catalyzed by the polypeptide are especially contemplated. Labeling of antibodies, antibody fragments, small molecules, and binding of the labeled entity is a technique that is well known in the art, and all known methods are generally suitable for use in conjunction with methods contemplated herein. Furthermore, the probe need not be limited to a fluorescein labeled antibody, and alternative probes include antibody fragments (e.g., Fab, Fab', scFab, etc.). General guidance regarding such techniques can be found in, e.g., Bancroft *et al.*, *Theory and Practice of Histological Techniques*, Churchill Livingstone, 1982, and Ausubel *et al.*, *supra*.

Still further contemplated variations include substitution of one or more atoms or chemical groups in the sequence with a radioactive atom or group. For example, where cDNAs are employed as a hybridization-specific probes, a fluorophor or enzyme (e.g., β-galactosidase for generation of a dye, or luciferase for generation of luminescence) may be coupled to the sequence to identify position and/or quantity of a complementary sequence. Alternatively, where contemplated cDNA molecules are utilized for affinity isolation procedures, the cDNA may be coupled to a molecule that is known to have a high-affinity (i.e., $K_d<10^{-4}mol^{-1}$) partner, such as biotin, or an oligo-histidyl tag. In another example, one or more phosphate groups may be exchanged for a radioactive phosphate group with a ^{32}P or ^{33}P isotope to assist in detection and quantification, where the radiolabeled cDNA is employed as a hybridization probe.

Diagnostic Kits

The invention also provides for a diagnostic test kit. For example, a diagnostic test kit can include antibodies to a STAMP2 polypeptide and components for detecting, and more preferably evaluating, binding between the antibodies and the STAMP2 polypeptide. For detection, either the antibody or the STAMP2 polypeptide is labeled, and either the antibody or the STAMP2 polypeptide is substrate-bound, such that STAMP2 polypeptide -antibody interaction can be established by determining the amount of label attached to the substrate following binding between the antibody and STAMP2 polypeptide. A conventional ELISA is a common, art-known method for detecting antibody-substrate interaction and can be provided with the kit of the invention. STAMP2 polypeptides can be detected in virtually any bodily fluid including, but not limited to urine, blood, semen, serum, plasma, saliva, amniotic fluid, or cerebrospinal fluid.

The invention also provides for a diagnostic test kit that includes a *STAMP2* nucleic acid probe or primer that can be used to detect and determine levels of STAMP2 nucleic acids or nucleic acids encoding a STAMP2 polypeptide. A kit that determines an alteration in the level of a *STAMP2* polypeptide or STAMP2 nucleic acid relative to a reference, such as the level present in a normal control, is useful as a diagnostic kit in the methods of the invention. Probes or primers useful in the diagnostic test kits can specifically bind to or hybridize to *STAMP2* nucleic acid molecules and, preferably, have greater than 45% sequence identity, more preferably at least 55-75% sequence identity, still more preferably at least 75-85% sequence identity, yet more preferably at least 85-95% sequence identity, and most preferably 96%, 97%, 98%, 99% or 100% sequence identity to a STAMP2 nucleic acid sequence, or fragment thereof. Probes can be detectably-labeled, either radioactively or non-radioactively, by methods that are well-known to those skilled in the art. Probes can be 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or more nucleotides in length. Probes can bind within the coding region of the STAMP2 nucleic acid molecule or outside the coding region (e.g., in the introns, or 5' or 3' non coding regions).

A probe or primer can be "detectably-labeled" using methods well known in the art including, without limitation, radioactive labeling (e.g., with an isotope, such as ^{32}P or ^{35}S) and nonradioactive labeling (e.g., with a fluorescent label, such as fluorescein, or by generating a construct containing green fluorescent protein (GFP)).

Desirably, the kit will contain instructions for the use of the kit. In one example, the kit contains instructions for the use of the kit for the diagnosis of a disorder of the prostate or testis, or the propensity to develop such a condition. In yet another example, the kit contains instructions for the use of the kit to monitor therapeutic treatment or dosage regimens.

Therapeutic Methods

The invention includes methods of treating or preventing disorders of the prostate or testis. In considering various therapies, it is understood that such therapies are, preferably, targeted to the affected or potentially affected organs, for example, the prostate or the testis. Reagents that are used to modulate STAMP2 expression or biological activity can include, without limitation, full length STAMP2 polypeptides or nucleic acids, which can be used, for example, to replace a mutant STAMP2 protein; mutants or fragments of STAMP2 polypeptides or nucleic acids; STAMP2 cDNA, mRNA, antisense RNA, or STAMP2 directed siRNA; STAMP2 antibodies; and any compound that modulates STAMP2 polypeptide or nucleic acid molecule biological activity, expression, or stability.

Therapeutic nucleobase oligomers that inhibit STAMP2 expression

Treatment or prevention of diseases resulting from a STAMP2 gene or polypeptide defect is accomplished, for example, by decreasing STAMP2 gene or protein levels or biological activity through the use of STAMP2 antisense, RNAi, or antibodies, or any additional compounds that can reduce the levels of STAMP2.

The present invention also features the use of nucleobase oligomers to downregulate expression of a STAMP2 nucleic acid or polypeptide. In one example, the nucleobase oligomer is an antisense nucleobase oligomer. By binding to the complementary nucleic acid sequence (the sense or coding strand), antisense nucleobase oligomers are able to inhibit protein expression presumably through the enzymatic cleavage of the RNA strand by RNAse H. Antisense-based strategies can be employed to explore STAMP2 gene function and as a basis for therapeutic drug design. These strategies are based on the principle that sequence-

specific suppression of gene expression (via transcription or translation) can be achieved by intracellular hybridization between genomic DNA or mRNA and a complementary antisense species. The formation of a hybrid RNA duplex interferes with transcription of the target STAMP2-encoding genomic DNA molecule, or processing, transport, translation, or stability of the target STAMP2 mRNA molecule.

Preferably, the antisense nucleobase oligomer is capable of reducing expression of a STAMP2 polypeptide or nucleic acid in a cell that expresses increased levels of that protein. Preferably the decrease in protein expression is at least 10% relative to cells treated with a control nucleobase oligomer, more preferably 25%, and most preferably 50%, 60%, 70%, 80%, 90% or greater. Methods for selecting and preparing antisense nucleobase oligomers are well known in the art.

Antisense strategies can be delivered by a variety of approaches. For example, antisense oligonucleotides or antisense RNA can be directly administered (e.g., to the affected prostate or testis tissue or by intravenous injection) to a subject in a form that allows uptake into cells. Alternatively, viral or plasmid vectors that encode antisense RNA (or antisense RNA fragments) can be introduced into a cell *in vivo* or *ex vivo*. Antisense effects can be induced by control (sense) sequences; however, the extent of phenotypic changes are highly variable. Phenotypic effects induced by antisense effects are based on changes in criteria such as protein levels, protein activity measurement, and target mRNA levels.

For example, *STAMP2* gene therapy can also be accomplished by direct administration of antisense *STAMP2* mRNA to a cell that is expected to be adversely affected by the expression of wild-type or mutant STAMP2 polypeptides. The antisense *STAMP2* mRNA can be produced and isolated by any

standard technique, but is most readily produced by *in vitro* transcription using an antisense *STAMP2* cDNA under the control of a high efficiency promoter (e.g., the T7 promoter). Administration of antisense *STAMP2* mRNA to cells can be carried out by any of the methods for direct nucleic acid molecule administration described above.

The present invention also features the use of RNA interference (RNAi) to inhibit expression of STAMP2. RNA interference (RNAi) is a post-transcriptional gene silencing (PTGS) mechanism in which double-stranded RNA (dsRNA) corresponding to a gene or mRNA of interest is introduced into an organism resulting in the degradation of the corresponding mRNA. In the RNAi reaction, both the sense and anti-sense strands of a dsRNA molecule are processed into small RNA fragments or segments ranging in length from 21 to 23 nucleotides (nt) and having 2-nucleotide 3' tails. Alternatively, synthetic dsRNAs, which are 21 to 23 nt in length and have 2-nucleotide 3' tails, can be synthesized, purified and used in the reaction. These 21 to 23 nt dsRNAs are known as "guide RNAs" or "short interfering RNAs" (siRNAs).

The siRNA duplexes then bind to a nuclease complex composed of proteins that target and destroy endogenous mRNAs having homology to the siRNA within the complex. Although the identity of the proteins within the complex remains unclear, the function of the complex is to target the homologous mRNA molecule through base pairing interactions between one of the siRNA strands and the endogenous mRNA. The mRNA is then cleaved approximately 12 nt from the 3' terminus of the siRNA and degraded. In this manner, specific genes can be targeted and degraded, thereby resulting in a loss of protein expression from the targeted gene.

The specific requirements and modifications of dsRNA are described in PCT Publication No. WO01/75164 (incorporated herein by reference). While

dsRNA molecules can vary in length, it is most preferable to use siRNA molecules which are 21- to 23- nucleotide dsRNAs with characteristic 2- to 3- nucleotide 3' overhanging ends typically either (2'-deoxy)thymidine or uracil. The siRNAs typically comprise a 3' hydroxyl group. Single stranded siRNA as well as blunt ended forms of dsRNA can also be used. In order to further enhance the stability of the RNA, the 3' overhangs can be stabilized against degradation. In one such embodiment, the RNA is stabilized by including purine nucleotides, such as adenosine or guanosine. Alternatively, substitution of pyrimidine nucleotides by modified analogs, e.g., substitution of uridine 2-nucleotide overhangs by (2'-deoxy)thymide is tolerated and does not affect the efficiency of RNAi. The absence of a 2' hydroxyl group significantly enhances the nuclease resistance of the overhang in tissue culture medium.

Alternatively siRNA can be prepared using standard procedures for *in vitro* transcription of RNA and dsRNA annealing procedures as described in Elbashir et al. (*Genes & Dev.*, 15:188-200, 2001). siRNAs are also obtained as described in Elbashir et al. by incubation of dsRNA that corresponds to a sequence of the target gene in a cell-free Drosophila lysate from syncytial blastoderm Drosophila embryos under conditions in which the dsRNA is processed to generate siRNAs of about 21 to about 23 nucleotides, which are then isolated using techniques known to those of skill in the art. For example, gel electrophoresis can be used to separate the 21-23 nt RNAs and the RNAs can then be eluted from the gel slices. In addition, chromatography (e.g., size exclusion chromatography), glycerol gradient centrifugation, and affinity purification with antibody can be used to isolate the 21 to 23 nt RNAs.

In the RNAi reaction, both the sense and anti-sense strands of a dsRNA molecule are processed into small RNA fragments or segments ranging in length from 18 to 25 nucleotides, preferably 21 to 23 nucleotides (nt), and having 2-

nucleotide 3' tails. Alternatively, synthetic dsRNAs, which are 21 to 23 nt in length and have 2-nucleotide 3' tails, can be synthesized, purified and used in the reaction. These 21 to 23 nt dsRNAs are known as "guide RNAs" or "short interfering RNAs" (siRNAs). dsRNAs or siRNAs that are useful in the present invention are substantially complementary (e.g., at least 85%, 90%, 95%, 99%, or more) to at least 18, 19, 20, 21, 22, 23, 24, or 25 consecutive nucleotides of a *STAMP2* nucleic acid. In the present invention, the dsRNA, or siRNA, is complementary to the mRNA sequence of *STAMP2* mRNA, or any fragment thereof, and can reduce or inhibit expression of STAMP2. Preferably, the decrease in STAMP2 protein expression is at least 10% relative to untreated cells or cells treated with a control dsRNA or siRNA, more preferably 25%, and most preferably at least 50%, 60%, 70%, 80%, 90% or greater.

A variety of methods are available for transfection, or introduction, of dsRNA or oligonucleotides into mammalian cells. For example, there are several commercially available transfection reagents including but not limited to: TransIT-TKO™ (Mirus, Cat. # MIR 2150), Transmessenger™ (Qiagen, Cat. # 301525), and Oligofectamine™ (Invitrogen, Cat. # MIR 12252-011). Protocols for each transfection reagent are available from the manufacturer.

In the present invention, the nucleobase oligomers used include any modification that enhances the stability or function of the nucleic acid in any way. Examples include modifications to the phosphate backbone, the internucleotide linkage, or to the sugar moiety. Examples of modifications that may be used in the nucleobase oligomers of the invention, can be found in U.S. Patent Application Publication Nos. 20030114412, paragraphs [0030] to [0046] and 20030114407, paragraphs [0036] to [0055], and 20030190659, paragraphs [0083] to [0106].

Therapeutic STAMP2 nucleic acids and polypeptides

In subjects where a defect or mutation is detected in the STAMP2 gene or polypeptide, for example using the diagnostic methods described herein, treatment or prevention of disorders of the prostate or testis can be accomplished by replacing a mutant *STAMP2* gene with a normal *STAMP2* gene, modulating the biological activity of a mutant STAMP2 protein, or altering the levels of a mutant STAMP2 protein. It is also possible to correct a *STAMP2* gene defect by modifying the physiological pathway (e.g., an intracellular trafficking or secretory pathway or cell proliferation pathway) in which the STAMP2 protein participates.

To replace a mutant or defective protein with normal protein, or to add protein to cells that do not express sufficient or normal STAMP2 protein, it may be necessary to obtain large amounts of pure STAMP2 protein from cultured cell systems in which the protein is expressed (see below). Delivery of the protein to the affected tissue can then be accomplished using appropriate packaging or administrating systems. For the therapeutic approaches that involve administration of recombinant STAMP2 polypeptide, STAMP2 can be administered either directly to the site of a potential or actual disease-affected tissue (for example, by injection) or systemically (for example, by any conventional recombinant protein administration technique). The dosage of STAMP2 depends on a number of factors, including the size and health of the individual patient, but, generally, between 0.001 mg/kg and 10 mg/kg, inclusive, are administered per day to an adult in any pharmaceutically acceptable formulation.

Gene therapy is another therapeutic approach for replacing a mutant or defective gene with a normal gene. Nucleic acid molecules encoding wild type STAMP2 proteins can be delivered to cells that lack sufficient, normal STAMP2 expression or biological activity (e.g., cells carrying mutations in *STAMP2* genes).

The nucleic acid molecules must be delivered to those cells in a form in which they can be taken up by the cells and so that levels of normal STAMP2 polypeptide, sufficient to provide effective STAMP2 function, can be produced. Alternatively, for some *STAMP2* mutations, it may be possible to slow the progression of the resulting disease or to modulate STAMP2 activity by introducing another copy of a homologous gene bearing a second mutation in that gene, to alter the mutation, or to use another gene to block any negative effect.

Modes for delivering nucleic acids

For any of the nucleic acid applications described herein, standard methods for administering nucleic acids can be used. For example, to simplify the manipulation and handling of the therapeutic nucleic acids, the STAMP2 nucleic acid is preferably inserted into a cassette where it is operably linked to a promoter. The promoter must be capable of driving expression of the nucleic acid in the desired target host cell. The selection of appropriate promoters can readily be accomplished. Preferably, one would use a high expression promoter. An example of a suitable promoter is the 763-base-pair cytomegalovirus (CMV) promoter. The Rous sarcoma virus (RSV) (Davis, et al., *Hum. Gene Ther.* 4:151-159, 1993) and mouse mammary tumor virus (MMTV) promoters may also be used. Certain proteins can be expressed using their native promoter. Other elements that can enhance expression can also be included (e.g., enhancers or a system that results in high levels of expression such as a tat gene and tar element). The enhancers used can include, without limitation, those that are characterized as tissue- or cell-specific enhancers. Alternatively, if a *STAMP2* genomic clone is used as a therapeutic construct (such clones can be identified by hybridization with *STAMP2* cDNA, described above), regulation can be mediated by the cognate regulatory sequences, or, if desired, by regulatory sequences derived from a

heterologous source, including any of the promoters or regulatory elements described above.

The recombinant vector can be a plasmid vector such as pUC118, pBR322, or other known plasmid vectors, that includes, for example, an *E. coli* origin of replication (see, Sambrook, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory press, 1989). The plasmid vector may also include a selectable marker such as the β lactamase gene for ampicillin resistance, provided that the marker polypeptide does not adversely affect the metabolism of the organism being treated. The cassette can also be bound to a nucleic acid binding moiety in a synthetic delivery system, such as the system disclosed in PCT Publication No. WO95/22618.

The nucleic acid can be introduced into the cells by any means appropriate for the vector employed. Many such methods are well known in the art (Sambrook et al., *supra*, and Watson et al., "Recombinant DNA", Chapter 12, 2d edition, Scientific American Books, 1992). Recombinant vectors can be transferred by methods such as calcium phosphate precipitation, electroporation, liposome-mediated transfection, gene gun, microinjection, viral capsid-mediated transfer, polybrene-mediated transfer, or protoplast fusion. For a review of the procedures for liposome preparation, targeting and delivery of contents, see Mannino and Gould-Fogerite, (*Bio Techniques*, 6:682-690, 1988), Felgner and Holm, (*Bethesda Res. Lab. Focus*, 11:21, 1989) and Maurer (*Bethesda Res. Lab. Focus,* 11:25, 1989).

Gene therapy can be performed *in vivo* or *ex vivo* by transfecting a cell line and then delivering the cells to the subject. Transducing retroviral, adenoviral, and adeno-associated viral vectors can be used for somatic cell gene therapy, especially because of their high efficiency of infection and stable integration and expression (see, e.g., Cayouette *et al.*, *Human Gene Therapy* 8:423-430, 1997;

Kido *et al., Current Eye Research* 15:833-844, 1996; Bloomer *et al., Journal of Virology* 71:6641-6649, 1997; Naldini *et al., Science* 272:263-267, 1996; and Miyoshi *et al., Proc. Natl. Acad. Sci., USA* 94:10319-1032, 1997). For example, the full length *STAMP2* gene, or a fragment thereof, can be cloned into a retroviral vector and expression can be driven from its endogenous promoter, from the retroviral long terminal repeat, or from a promoter specific for a target cell type of interest. Other viral vectors that can be used include, for example, vaccinia virus, bovine papilloma virus, or a herpes virus, such as Epstein-Barr Virus (also see, for example, the vectors of Miller, *Human Gene Therapy* 15-14, 1990; Friedman, *Science* 244:1275-1281, 1989; Eglitis *et al., BioTechniques* 6:608-614, 1988; Tolstoshev *et al., Current Opinion in Biotechnology* 1:55-61, 1990; Sharp, *The Lancet* 337:1277-1278, 1991; Cornetta *et al., Nucleic Acid Research and Molecular Biology* 36:311-322, 1987; Anderson, *Science* 226:401-409, 1984; Moen, *Blood Cells* 17:407-416, 1991; or Miller *et al., Biotechnology* 7:980-990, 1989). Retroviral and lentiviral vectors are particularly well developed and have been used in clinical settings (Rosenberg *et al., N. Engl. J. Med* 323:370, 1990; Anderson *et al.*, U.S. Patent No. 5,399,346).

Transfer of the recombinant vector (either plasmid vector or viral vectors) can be accomplished through direct injection into the prostate or testis tissue or more generally via intravenous delivery. Transplantation of normal genes into the affected tissues of a patient can also be accomplished by transferring a normal *STAMP2* gene into a cultivatable cell type *ex vivo*, after which the cell (or its descendants) is injected into a targeted tissue. Another strategy for inhibiting STAMP2 function using gene therapy involves intracellular expression of an anti-STAMP2 antibody or a portion of an anti-STAMP2 antibody. For example, the gene (or gene fragment) encoding a monoclonal antibody that specifically binds to

STAMP2 polypeptide and inhibits its biological activity is placed under the transcriptional control of a tissue-specific gene regulatory sequence.

Non-viral approaches can also be employed for the introduction of therapeutic DNA into cells predicted to be subject to diseases involving a STAMP2 disorder. For example, a *STAMP2* nucleic acid molecule or an antisense nucleic acid molecule can be introduced into a cell by lipofection (Felgner *et al.*, *Proc. Natl. Acad. Sci. USA* 84:7413, 1987; Ono *et al.*, *Neuroscience Letters* 17:259, 1990; Brigham *et al.*, *Am. J. Med. Sci.* 298:278, 1989; Staubinger *et al.*, *Methods in Enzymology* 101:512, 1983), asialoorosomucoid-polylysine conjugation (Wu *et al.*, *Journal of Biological Chemistry* 263:14621, 1988; Wu *et al.*, *Journal of Biological Chemistry* 264:16985, 1989), or, less preferably, micro-injection under surgical conditions (Wolff *et al.*, *Science* 247:1465, 1990).

For any of the above therapeutic methods, the therapies described herein can be administered before, during, or after the onset of the disease phenotype. In addition, compounds shown to modulate STAMP2 polypeptide or nucleic acid molecule expression or biological activity are administered to patients diagnosed with potential or actual diseases by any standard dosage and route of administration. In one example where a mutation in STAMP2 is detected prior to onset of disease, and known to be associated with disorders of the prostate or testis, gene therapy using a STAMP2 mRNA expression construct is undertaken to reverse or prevent the gene defect prior to the development of the full course of the disease.

The therapeutic methods of the invention are, in some cases, targeted to prenatal treatment. For example, a fetus found to have a STAMP2 mutation is administered a gene therapy vector including a normal *STAMP2* gene, or administered a normal STAMP2 protein. Such treatment may be required only for a short period of time, or may, in some form, be required throughout such a

patient's lifetime. Any continued need for treatment, however, is determined using, for example, the diagnostic methods described above. Also as discussed above, STAMP2 polypeptide or nucleic acid molecule abnormalities may be associated with diseases in adults, and thus, adults are subject to the therapeutic methods of the invention as well.

Additionally, STAMP2 polypeptides may be used to stimulate an immune system to assist in generating immunity against, for example, prostate cancer cells.

Assays for gene and protein expression

The following methods can be used to evaluate protein or gene expression and determine efficacy for any of the above-mentioned methods for increasing or decreasing the expression of STAMP2 nucleic acids or polypeptides.

A sample of bodily fluid (e.g., semen, blood, serum, plasma, urine, amniotic fluid, and cerebrospinal fluid) or a tissue sample (e.g., prostate or testis) from the subject is measured for levels of STAMP2 polypeptide, using methods such as, immunohistochemistry, ELISA, western blotting, or immunoassays using specific antibodies.

Subject samples from the subject can also be measured for levels of nucleic acid encoding a polypeptide of the invention. There are several art-known methods to assay for gene expression. Some examples include the preparation of RNA from the subject sample and the use of the RNA for northern blotting, PCR based amplification, in situ hybridization or RNAse protection assays.

A positive result is considered an alteration of at least 20%, preferably 30%, more preferably at least 50%, and most preferably at least 60%, 70%, 80%, 90%, or more in the levels of a STAMP2 nucleic acid or polypeptide as compared to a reference sample. Generally, in subjects having a disorder associated with an increase in STAMP2 nucleic acid or polypeptide levels, the desired result after

therapy is a decrease in the level of STAMP2 nucleic acid or polypeptide expression.

Therapeutic Antibodies

The present invention provides antibodies that bind specifically to the STAMP2 polypeptide. The antibodies are used to neutralize or inhibit the activity of STAMP2. Antibodies that inhibit the activity of a STAMP2 polypeptide can also be useful in preventing or slowing the development of a disease caused by inappropriate expression of a wild type or mutant *STAMP2* gene. In one example, the antibodies of the invention may be utilized to localize and locally quantify disease-specific markers in prostate or testis tissue sections, e.g, in prostate or testicular cancer. Preferred antibodies of the invention specifically bind to amino acids 1 to 20, 70 to 82, 87 to 97, 330 to 347, 400 to 428, or 445 to 459 of STAMP2 (SEQ ID NO: 2).

Methods for the preparation and use of antibodies for therapeutic purposes are described in several patents including U.S. Patent Numbers 6,054,297; 5,821,337; 6,365,157; and 6,165,464 and are incorporated herein by reference. Antibodies can be polyclonal or monoclonal; monoclonal antibodies are preferred.

Monoclonal antibodies, particularly those derived from rodents including mice, have been used for the treatment of various diseases; however, there are limitations to their use including the induction of a human anti-mouse immunoglobulin response that causes rapid clearance and a reduction in the efficacy of the treatment. For example, a major limitation in the clinical use of rodent monoclonal antibodies is an anti-globulin response during therapy (Miller et al., *Blood*, 62:988-995 1983; Schroff et al., *Cancer Res.*, 45:879-885, 1985).

The art has attempted to overcome this problem by constructing "chimeric" antibodies in which an animal antigen-binding variable domain is coupled to a

human constant domain (U.S. Pat. No. 4,816,567; Morrison et al., *Proc. Natl. Acad. Sci. USA,* 81:6851-6855, 1984; Boulianne et al., *Nature,* 312:643-646, 1984; Neuberger et al., *Nature,* 314:268-270, 1985). The production and use of such chimeric antibodies are described below.

A cocktail of the monoclonal antibodies of the present invention can be used as an effective treatment for disorders of the prostate or testis. The cocktail may include as few as two, three, or four different antibodies or as many as six, eight, or ten different antibodies. In addition, the antibodies of the present invention can be combined with anti-cancer therapies that are known in the art (e.g., chemotherapy, radiotherapy).

Monoclonal antibodies that specifically bind to STAMP2, or fragments thereof, may be produced by methods known in the art. These methods include the immunological method described by Kohler and Milstein (*Nature,* 256: 495-497, 1975) and Campbell ("Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas" in Burdon et al., Eds., Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam, 1985), as well as by the recombinant DNA method described by Huse et al. (*Science,* 246, 1275-1281, 1989).

Monoclonal antibodies may be prepared from supernatants of cultured hybridoma cells or from ascites induced by intra-peritoneal inoculation of hybridoma cells into mice. The hybridoma technique described originally by Kohler and Milstein (*Eur. J. Immunol,* 6, 511-519, 1976) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens.

The route and schedule of immunization of the host animal or cultured antibody-producing cells therefrom are generally in keeping with established and conventional techniques for antibody stimulation and production. Typically, mice

are used as the test model, however, any mammalian subject including human subjects or antibody producing cells therefrom can be manipulated according to the processes of this invention to serve as the basis for production of mammalian, including human, hybrid cell lines.

After immunization, immune lymphoid cells are fused with myeloma cells to generate a hybrid cell line that can be cultivated and subcultivated indefinitely, to produce large quantities of monoclonal antibodies. For purposes of this invention, the immune lymphoid cells selected for fusion are lymphocytes and their normal differentiated progeny, taken either from lymph node tissue or spleen tissue from immunized animals. The use of spleen cells is preferred, since they offer a more concentrated and convenient source of antibody producing cells with respect to the mouse system. The myeloma cells provide the basis for continuous propagation of the fused hybrid. Myeloma cells are tumor cells derived from plasma cells. Murine myeloma cell lines can be obtained, for example, from the American Type Culture Collection (ATCC; Manassas, VA). Human myeloma and mouse-human heteromyeloma cell lines have also been described (Kozbor et al., *J. Immunol.*, 133:3001-3005, 1984; Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, pp. 51-63, 1987).

The hybrid cell lines can be maintained *in vitro* in cell culture media. Once the hybridoma cell line is established, it can be maintained on a variety of nutritionally adequate media such as hypoxanthine-aminopterin-thymidine (HAT) medium. Moreover, the hybrid cell lines can be stored and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely with resumed synthesis and secretion of monoclonal antibody. The secreted antibody is recovered from tissue culture supernatant by conventional methods such as precipitation, ion exchange chromotography, affinity chromatography, or the like.

The antibody may be prepared in any mammal, including mice, rats, rabbits, goats, and humans. The antibody may be a member of one of the following immunoglobulin classes: IgG, IgM, IgA, IgD, or IgE, and the subclasses thereof, and preferably is an IgG antibody.

While the preferred animal for producing monoclonal antibodies is mouse, the invention is not so limited; in fact, human antibodies may be used and may prove to be preferable. Such antibodies can be obtained by using human hybridomas (Cole et al., "Monoclonal Antibodies and Cancer Therapy", Alan R. Liss Inc., p. 77-96, 1985). In the present invention, techniques developed for the production of chimeric antibodies by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule can be used (Morrison et al., *Proc. Natl. Acad. Sci.* 81, 6851-6855, 1984; Neuberger et al., *Nature* 312, 604-608, 1984; Takeda et al., *Nature* 314, 452-454, 1985); such antibodies are within the scope of this invention and are described below.

As another alternative to the cell fusion technique, Epstein-Barr virus (EBV) immortalized B cells are used to produce the monoclonal antibodies of the present invention (Crawford D. et al., *J. of Gen. Virol.,* 64:697-700, 1983; Kozbor and Roder, *J. Immunol.*, 4:1275-1280, 1981; Kozbor et al., *Methods in Enzymology,* 121:120-140, 1986). In general, the procedure consists of isolating Epstein-Barr virus from a suitable source, generally an infected cell line, and exposing the target antibody secreting cells to supernatants containing the virus. The cells are washed, and cultured in an appropriate cell culture medium. Subsequently, virally transformed cells present in the cell culture can be identified by the presence of the Epstein-Barr viral nuclear antigen, and transformed antibody secreting cells can be identified using standard methods known in the art.

Other methods for producing monoclonal antibodies, such as recombinant DNA, are also included within the scope of the invention.

Preparation of immunogens

STAMP2 polypeptides may be used alone as an immunogen, or may be attached to a carrier protein or to other objects, such as sepharose beads. Preferred STAMP2 fragments for use as an immunogen include amino acids 1 to 20, amino acids 70 to 82, amino acids 87 to 97, amino acids 330 to 347, amino acids 400 to 428, or amino acids 445 to 459 of STAMP2 (SEQ ID NO: 2). STAMP2 polypeptides may be purified from cells known to express the endogenous protein such as prostate cells (e.g., LNCaP cells). Additionally, nucleic acid molecules that encode any of the polypeptides of the invention, or portions thereof, can be inserted into known vectors for expression in host cells using standard recombinant DNA techniques. Suitable host cells for protein expression include baculovirus cells (e.g., Sf9 cells), bacterial cells (e.g., *E. coli*), and mammalian cells (e.g., NIH3T3 cells).

In addition, peptides can be synthesized and used as immunogens. The methods for making antibody to peptides are well known in the art and generally require coupling the peptide to a suitable carrier molecule, such as serum albumin. Peptides can be any length, preferably 10 amino acids or greater, more preferably 25 amino acids or greater, and most preferably 40, 50, 60, 70, 80, or 100 amino acids or greater. Preferably, the amino acid sequences are at least 80%, more preferably 85%, and, most preferably 95% to 100% identical to the sequence of a *STAMP2* nucleic acid sequence. Additionally preferred peptides are at least 80%, more preferably 85%, and most preferably 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of amino acids 1 to 20, 70 to 82, 87 to 97, 330 to 347, 400 to 428, or 445 to 459 of STAMP2. The peptides can be commercially obtained

or made using techniques well known in the art, such as, for example, the Merrifield solid-phase method (*Science*, 232:341-347, 1985). The procedure may use commercially available synthesizers such as a Biosearth 9500 automated peptide machine, with cleavage of the blocked amino acids being achieved with hydrogen fluoride, and the peptides purified by preparative HPLC using a Waters Delta Prep 3000 instrument, on a 15-20 μm Vydac C4 PrepPAK column.

Functional equivalents of antibodies

The invention also includes functional equivalents of the antibodies described in this specification. Functional equivalents include polypeptides with amino acid sequences substantially identical to the amino acid sequence of the variable or hypervariable regions of the antibodies of the invention. Functional equivalents have binding characteristics comparable to those of the antibodies, and include, for example, chimerized, humanized and single chain antibodies as well as fragments thereof. Methods of producing such functional equivalents are disclosed, for example, in PCT Publication No. WO93/21319; European Patent Application No. 239,400; PCT Publication No. WO89/09622; European Patent Application No. 338,745; European Patent Application No. 332424; and U.S. Patent No. 4,816,567; each of which is herein incorporated by reference.

Chimerized antibodies preferably have constant regions derived substantially or exclusively from human antibody constant regions and variable regions derived substantially or exclusively from the sequence of the variable region from a mammal other than a human. Such humanized antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Methods for humanizing non-human antibodies are well known in the art (for reviews see

Vaswani and Hamilton, *Ann Allergy Asthma Immunol.*, 81:105-119, 1998 and Carter, *Nature Reviews Cancer*, 1:118-129, 2001). Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the methods known in the art (Jones et al., *Nature*, 321:522-525, 1986; Riechmann et al., *Nature,* 332:323-329, 1988; and Verhoeyen et al., *Science*, 239:1534-1536 1988), by substituting rodent CDRs or other CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species (see for example, U.S. Patent No. 4,816,567). In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies (Presta, *Curr. Op. Struct. Biol.*, 2:593-596, 1992).

Additional methods for the preparation of humanized antibodies can be found in U.S. Patent Nos. 5,821,337, 6,054,297, 6,639,055, and Carter, (*supra*) which are all incorporated herein by reference. The humanized antibody is selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG_1, IgG_2, IgG_3, and IgG_4. Where cytotoxic activity is not needed, such as in the present invention, the constant domain is preferably of the IgG_2 class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries (Marks et al., *J. Mol. Biol.*, 222:581-597,

1991 and Winter et al. *Annu. Rev. Immunol.*, 12:433-455, 1994). The techniques of Cole et al. and Boerner et al. are also useful for the preparation of human monoclonal antibodies (Cole et al., *supra*; Boerner et al., *J. Immunol.*, 147: 86-95, 1991).

Suitable mammals other than a human include any mammal from which monoclonal antibodies may be made. Examples of mammals other than a human include, for example a rabbit, rat, mouse, horse, goat, or primate; a mouse is preferred.

Functional equivalents of antibodies also include single-chain antibody fragments, also known as single-chain antibodies (scFvs). Single-chain antibody fragments are recombinant polypeptides which typically bind antigens or receptors; these fragments contain at least one fragment of an antibody variable heavy-chain amino acid sequence (V_H) tethered to at least one fragment of an antibody variable light-chain sequence (V_L) with or without one or more interconnecting linkers. Such a linker may be a short, flexible peptide selected to assure that the proper three-dimensional folding of the V_L and V_H domains occurs once they are linked so as to maintain the target molecule binding-specificity of the whole antibody from which the single-chain antibody fragment is derived. Generally, the carboxyl terminus of the V_L or V_H sequence is covalently linked by such a peptide linker to the amino acid terminus of a complementary V_L and V_H sequence. Single-chain antibody fragments can be generated by molecular cloning, antibody phage display library or similar techniques. These proteins can be produced either in eukaryotic cells or prokaryotic cells, including bacteria.

Single-chain antibody fragments contain amino acid sequences having at least one of the variable regions or CDRs of the whole antibodies described in this specification, but are lacking some or all of the constant domains of those antibodies. These constant domains are not necessary for antigen binding, but

constitute a major portion of the structure of whole antibodies. Single-chain antibody fragments may therefore overcome some of the problems associated with the use of antibodies containing part or all of a constant domain. For example, single-chain antibody fragments tend to be free of undesired interactions between biological molecules and the heavy-chain constant region, or other unwanted biological activity. Additionally, single-chain antibody fragments are considerably smaller than whole antibodies and may therefore have greater capillary permeability than whole antibodies, allowing single-chain antibody fragments to localize and bind to target antigen-binding sites more efficiently. Also, antibody fragments can be produced on a relatively large scale in prokaryotic cells, thus facilitating their production. Furthermore, the relatively small size of single-chain antibody fragments makes them less likely than whole antibodies to provoke an immune response in a recipient.

Functional equivalents further include fragments of antibodies that have the same or comparable binding characteristics to those of the whole antibody. Such fragments may contain one or both Fab fragments or the $F(ab')_2$ fragment. Preferably the antibody fragments contain all six CDRs of the whole antibody, although fragments containing fewer than all of such regions, such as three, four or five CDRs, are also functional.

Further, the functional equivalents may be or may combine members of any one of the following immunoglobulin classes: IgG, IgM, IgA, IgD, or IgE, and the subclasses thereof.

Preparation of Functional Equivalents of Antibodies

Equivalents of antibodies are prepared by methods known in the art. For example, fragments of antibodies may be prepared enzymatically from whole antibodies. Preferably, equivalents of antibodies are prepared from DNA

encoding such equivalents. DNA encoding fragments of antibodies may be prepared by deleting all but the desired portion of the DNA that encodes the full-length antibody.

DNA encoding chimerized antibodies may be prepared by recombining DNA substantially or exclusively encoding human constant regions and DNA encoding variable regions derived substantially or exclusively from the sequence of the variable region of a mammal other than a human. DNA encoding humanized antibodies may be prepared by recombining DNA encoding constant regions and variable regions other than the CDRs derived substantially or exclusively from the corresponding human antibody regions and DNA encoding CDRs derived substantially or exclusively from a mammal other than a human.

Suitable sources of DNA molecules that encode fragments of antibodies include cells, such as hybridomas, that express the full-length antibody. The fragments may be used by themselves as antibody equivalents, or may be recombined into equivalents, as described above.

The DNA deletions and recombinations described in this section may be carried out by known methods, such as those described in the published patent applications listed above.

Antibody Screening and Selection

Monoclonal antibodies are isolated and purified using standard art-known methods. For example, antibodies can be screened using standard art-known methods such as ELISA or western blot analysis.

Therapeutic Uses of Antibodies

When used *in vivo* for the treatment or prevention of a disorder of the prostate or testis, the antibodies of the subject invention are administered to the subject in therapeutically effective amounts. Preferably, the antibodies are

administered parenterally or intravenously by continuous infusion. The dose and dosage regimen depends upon the severity of the disease, and the overall health of the subject. The amount of antibody administered is typically in the range of about 0.001 to about 10 mg/kg of subject weight, preferably 0.01 to about 5 mg/kg of subject weight.

For parenteral administration, the antibodies are formulated in a unit dosage injectable form (solution, suspension, emulsion) in association with a pharmaceutically acceptable parenteral vehicle. Such vehicles are inherently nontoxic, and non-therapeutic. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils and ethyl oleate may also be used. Liposomes may be used as carriers. The vehicle may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, e.g., buffers and preservatives. The antibodies typically are formulated in such vehicles at concentrations of about 1 mg/ml to 10 mg/ml.

Combination therapies

Optionally, a therapeutic of the invention may be administered in combination with any other standard anti-cancer therapy; such methods are known to the skilled artisan and include radiation therapy, chemotherapy, anti-cancer antibiotics, steroid hormones, or hormone antagonists, and therapeutic antibodies (e.g., Herceptin).

Modes of Administration

A STAMP2 polypeptide, nucleic acid molecule, or modulator is administered within a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form to patients or experimental animals. Also, conventional

pharmaceutical practice is employed to provide suitable formulations or compositions in which to administer neutralizing STAMP2 antibodies or STAMP2-inhibiting compounds (e.g., a STAMP2 antisense molecule, STAMP2 dsRNA molecule, or a STAMP2 dominant negative mutant) to patients suffering from a STAMP2 disease, such as prostate cancer, testicular cancer, benign hyperplasia of the prostate, or developmental defects of the prostate or testis. Administration can begin before or after the patient is symptomatic.

Any appropriate route of administration can be employed, for example, administration can be parenteral, intravenous, intra-arterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, inhalation to deep lung, aerosol, by suppositories, oral, or topical (e.g., by applying an adhesive patch carrying a formulation capable of crossing the dermis and entering the bloodstream). Preferably, the administration is local to the afflicted tissue, such as prostate or testis tissue. Therapeutic formulations can be in the form of liquid solutions or suspensions; for oral administration, formulations can be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols. Any of the above formulations may be a sustained-release formulation.

Methods that are well known in the art for making formulations are found, for example, in *Remington's Pharmaceutical Sciences*, *supra*. Formulations for parenteral administration can, for example, contain excipients; sterile water; or saline; polyalkylene glycols, such as polyethylene glycol; oils of vegetable origin; or hydrogenated napthalenes. Sustained-release, biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers can be used to control the release of the compounds. Other potentially useful parenteral delivery systems for STAMP2

modulatory compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation can contain excipients, for example, lactose, or can be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate, and deoxycholate, or can be oily solutions for administration in the form of nasal drops, or as a gel.

Screening Assays

As discussed above, the expression of a STAMP2 nucleic acid or polypeptide is increased in a subject having a disorder of the prostate or the testis. Based on these discoveries, compositions of the invention are useful for the high-throughput low-cost screening of candidate compounds to identify those that modulate the expression of a STAMP2 nucleic acid or polypeptide whose expression is altered in a subject having a disorder of the prostate or the testis.

Any number of methods are available for carrying out screening assays to identify new candidate compounds that alter the expression of a STAMP2 nucleic acid or polypeptide. In one working example, candidate compounds are added at varying concentrations to the culture medium of cultured cells expressing a STAMP2 nucleic acid sequence. These cells can then be used to screen for new candidate compounds. Gene expression is then measured, for example, by microarray analysis, Northern blot analysis (Ausubel et al., *supra*), or RT-PCR, using any appropriate fragment prepared from the nucleic acid molecule as a hybridization probe. The level of gene expression in the presence of the candidate compound is compared to the level measured in a control culture medium lacking the candidate compound. A compound that promotes a decrease in the expression of a STAMP2 nucleic acid or polypeptide is considered useful in the invention;

such a molecule may be used, for example, as a therapeutic to treat a disorder of the prostate or testis.

In another working example, the effect of candidate compounds may be measured at the level of polypeptide production using the same general approach and standard immunological techniques, such as Western blotting or immunoprecipitation with an antibody specific for a STAMP2 polypeptide. For example, immunoassays may be used to detect or monitor the expression of at least one of the polypeptides of the invention in an organism. Polyclonal or monoclonal antibodies (produced as described above) that are capable of binding to STAMP2 polypeptide may be used in any standard immunoassay format (e.g., ELISA, western blot, or RIA assay) to measure the level of the polypeptide. In some embodiments, a compound that promotes a decrease in the expression or biological activity of a STAMP2 polypeptide is considered particularly useful. Again, such a molecule may be used, for example, as a therapeutic to delay, ameliorate, or treat a disorder of the prostate or testis, or the symptoms of a disorder of the prostate or testis, in a subject.

In yet another working example, candidate compounds may be screened for those that specifically bind to a STAMP2 polypeptide. The efficacy of such a candidate compound is dependent upon its ability to interact with such a polypeptide or a functional equivalent thereof. Such an interaction can be readily assayed using any number of standard binding techniques and functional assays (e.g., those described in Ausubel et al., *supra*). In one embodiment, a candidate compound may be tested *in vitro* for its ability to specifically bind a STAMP2 polypeptide.

In another working example, a STAMP2 nucleic acid is expressed as a transcriptional or translational fusion with a detectable reporter, and expressed in an isolated cell (e.g., mammalian or insect cell) under the control of a heterologous

promoter, such as an inducible promoter. The cell expressing the fusion protein is then contacted with a candidate compound, and the expression of the detectable reporter in that cell is compared to the expression of the detectable reporter in an untreated control cell. A candidate compound that decreases the expression of a STAMP2 detectable reporter is a compound that is useful for the treatment of a disorder of the prostate or testis. In preferred embodiments, the candidate compound alters the expression of a reporter gene fused to a nucleic acid or nucleic acid.

In one particular working example, a candidate compound that binds to a STAMP2 polypeptide may be identified using a chromatography-based technique. For example, a recombinant STAMP2 polypeptide may be purified by standard techniques from cells engineered to express the polypeptide (e.g., those described above) and may be immobilized on a column. A solution of candidate compounds is then passed through the column, and a compound specific for the STAMP2 polypeptide is identified on the basis of its ability to bind to the polypeptide and be immobilized on the column. To isolate the compound, the column is washed to remove non-specifically bound molecules, and the compound of interest is then released from the column and collected. Similar methods may be used to isolate a compound bound to a polypeptide microarray. Compounds isolated by this method (or any other appropriate method) may, if desired, be further purified (e.g., by high performance liquid chromatography). In addition, these candidate compounds may be tested for their ability to decrease the activity of a STAMP2 polypeptide. Compounds isolated by this approach may also be used, for example, as therapeutics to treat a disorder of the prostate or testis in a human subject. Compounds that are identified as binding to a STAMP2 polypeptide with an affinity constant less than or equal to 10 mM are considered particularly useful in the invention. Alternatively, any *in vivo* protein interaction detection system, for

example, any two-hybrid assay may be utilized to identify compounds or proteins that bind to a STAMP2 polypeptide.

Potential antagonists include organic molecules, peptides, peptide mimetics, polypeptides, nucleic acids, and antibodies that bind to a STAMP2 nucleic acid or polypeptide.

STAMP2 DNA sequences may also be used in the discovery and development of a therapeutic compound for the treatment of a disorder of the prostate or testis. The encoded protein, upon expression, can be used as a target for the screening of drugs. Additionally, the DNA sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences may be isolated by standard techniques (Ausubel et al., *supra*).

Optionally, compounds identified in any of the above-described assays may be confirmed as useful in an assay for compounds that decrease the biological activity of a STAMP2 polypeptide.

Small molecules of the invention preferably have a molecular weight below 2,000 daltons, more preferably between 300 and 1,000 daltons, and most preferably between 400 and 700 daltons. It is preferred that these small molecules are organic molecules.

Test compounds and extracts

In general, compounds capable of decreasing the activity of a STAMP2 nucleic acid or polypeptide are identified from large libraries of both natural product or synthetic (or semi-synthetic) extracts or chemical libraries or from polypeptide or nucleic acid libraries, according to methods known in the art. Those skilled in the field of drug discovery and development will understand that the precise source of test extracts or compounds is not critical to the screening

procedure(s) of the invention. Compounds used in screens may include known compounds (for example, known therapeutics used for other diseases or disorders). Alternatively, virtually any number of unknown chemical extracts or compounds can be screened using the methods described herein. Examples of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-based extracts, fermentation broths, and synthetic compounds, as well as modification of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries are commercially available from Brandon Associates (Merrimack, NH) and Aldrich Chemical (Milwaukee, WI). Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are commercially available from a number of sources, including Biotics (Sussex, UK), Xenova (Slough, UK), Harbor Branch Oceangraphics Institute (Ft. Pierce, FL), and PharmaMar, U.S.A. (Cambridge, MA). In addition, natural and synthetically produced libraries are produced, if desired, according to methods known in the art, e.g., by standard extraction and fractionation methods. Furthermore, if desired, any library or compound is readily modified using standard chemical, physical, or biochemical methods.

In addition, those skilled in the art of drug discovery and development readily understand that methods for dereplication (e.g., taxonomic dereplication, biological dereplication, and chemical dereplication, or any combination thereof) or the elimination of replicates or repeats of materials already known for their molt-disrupting activity should be employed whenever possible.

When a crude extract is found to decrease the activity of a STAMP2 nucleic acid or polypeptide, or to bind to a STAMP2 polypeptide, further fractionation of

the positive lead extract is necessary to isolate chemical constituents responsible for the observed effect. Thus, the goal of the extraction, fractionation, and purification process is the careful characterization and identification of a chemical entity within the crude extract that decreases the activity of a STAMP2 nucleic acid or polypeptide. Methods of fractionation and purification of such heterogeneous extracts are known in the art. If desired, compounds shown to be useful as therapeutics for the treatment of a disorder of the prostate or testis are chemically modified according to methods known in the art.

Examples

Example 1. Isolation and characterization of the *STAMP2* gene and mRNA.

In an attempt to identify genes that were similar to *STAMP1* (Korkmaz et al., *DNA Cell Biol.* 19:499-506, 2000), we identified a BAC clone (GenBank accession # AC003991) on human Chr7q21 that showed significant sequence similarity to *STAMP1* cDNA (Figure 1A). Computational exon/intron junction analysis of this locus and alignment of the full-length cDNA sequence revealed that there is a gene at this locus that is composed of five exons and four introns (Figure 1A). Given the similarity in both the gene structure, the predicted amino acid sequence (44% identity, 64% conservation), as well as the similarities in intracellular distribution to *STAMP1* (see below), we named this gene *STAMP2*. The *STAMP2* gene spans around 26 kb which is in part due to the extremely large size of intron 1 (22516 bp).

STAMP1 and *STAMP2* both lie on Chr7q, but are transcribed in opposite directions (Figure 1B). The *STAMP1* related gene, Six Transmembrane Epithelial Antigen of Prostate (STEAP) (Hubert et al., *Proc. Natl. Acad. Sci.* 96:14523-14528, 1999) is also in this locus and is transcribed in the same direction as

STAMP1. Thus, Chr7q contains a cluster of genes predicted to encode six transmembrane proteins.

We cloned the full-length cDNA for *STAMP2* from an R1881-induced LNCaP cDNA library using PCR. The cDNA and predicted amino acid sequence are presented in Figure 2. Because the *STAMP2* mRNA runs as a 4.0 kb band in a northern blot analysis (see below) and the cDNA we cloned is runs as a 2.3 kb including the poly (A) tail, we predict that *STAMP2* mRNA has approximately 1.7 kb of 5'-UTR sequences.

BLAST search of GenBank with the *STAMP2* cDNA revealed some homology (78% identity on the predicted amino acid level) to the previously described Tumor Necrosis Factor α-induced Adipose-related Protein (TIARP; Moldes et al., *J. Biol. Chem.* 276:33938-33946, 2001), a mouse protein which may have a role in adipocyte differentiation (Figure 3). In response to TNF-α stimulation, TIARP localizes to the plasma membrane suggesting that it may act as a channel or receptor on the mature adipocyte (Moldes et al., *supra*). In addition to TIARP and STAMP1, STAMP2 displays similarity to the rat protein pHyde (Steiner et al., *Cancer Res.* 60:4419-4425, 2000) (Figure 3). When pHyde is overexpressed, it causes apoptosis in prostate cancer cells, and the human homolog of pHyde, TSAP6, regulates apoptosis and the cell cycle through interactions with Nix and Myt1 kinase (Passer et al., *Proc. Natl. Acad. Sci.* 100:2284-2289, 2003). These data suggest that STAMP1, STAMP2, and pHyde/TSAP6 may be structurally, and possibly also functionally, related proteins.

In addition to the six transmembrane domains in the C-terminal half of the predicted STAMP2 sequence (Figure 2), a conserved domain search identified three motifs in the N-terminal domain of STAMP2. The first is a predicted dinucleotide-binding domain that is found in a number of bacterial proteins

(Deppenmeier, *Cell Mol. Life Sci.* 59:1513-1533, 2002). Second is an NADP oxidoreductase motif that is coenzyme F420-dependent, such as F420H2:NADP+ oxidoreductase found in archeabacteria (Warkentin et al., *EMBO J.* 20:6561-6569, 2001). Third is a motif that resembles pyrroline 5-carboxylate reductase, an enzyme that is involved in amino acid transport and metabolism (Phang, *Curr. Top. Cell Regul.* 25:91-132, 1985).

Example 2. *STAMP2* has a restricted tissue distribution.

We next determined the expression profile of *STAMP2* in various human tissues by northern analysis in which a multiple tissue northern blot was hybridized to the *STAMP2* cDNA probe. As shown in Figure 4A, *STAMP2* hybridized to a major mRNA species of 4.0 kb in placenta, lung, heart, and prostate, with substantially lower expression also seen in liver, skeletal muscle, pancreas, testis, and small intestine; there was no detectable expression in brain, kidney, spleen, colon or peripheral blood leukocytes. Hybridization with a glyceraldehyde 3-phosphate dehydrogenase (G3PDH) cDNA probe resulted in approximately similar signals in all lanes, except for the heart and skeletal muscle where *G3PDH* mRNA is known to be more abundant compared with other tissues.

Example 3. Characterization of *STAMP2* expression in cell lines and its androgen regulation.

Since one of the tissues in which *STAMP2* is highly expressed is the prostate, where androgen is a major hormonal stimulus for growth and for early stage prostate cancer (Huggins et al., *Arch. Surg.* 43:209-223, 1941), we assessed the possible androgen regulation of *STAMP2* by northern analysis in the androgen responsive prostate cancer cell line LNCaP (Horoszewicz et al., *Cancer Res.* 43:1809-1818, 1983). Cells were either left untreated or treated with the synthetic

androgen R1881 for 24 hours and harvested. Total RNA was isolated and used in Northern analysis with *STAMP2* cDNA as a probe. As shown in Figure 4B, *STAMP2* expression dramatically increased upon R1881 treatment of LNCaP cells. In contrast, there was no significant difference in the mRNA accumulation of *STAMP1* in the same samples, consistent with previous findings (Korkmaz et al., *supra*). Similar to *STAMP1*, there was no detectable expression of *STAMP2* in a number of prostate cancer cell lines that do not express the androgen receptor (PC-3, DU-145, CA-HPV10, PZ-HPV7, YPEN-1). In addition, *STAMP2* was not expressed in myotubes (C2), lung cancer cells (NCI-H661), breast cancer cells (MCF7, MCF7-LCC1, MCF7-LCC2, MB435), or cervical carcinoma cells (HeLa) (Figure 4B). Time course analysis of androgen treatment revealed that *STAMP2* expression began around six hours and increased through 48 hours (Figure 4C). Thus, expression of *STAMP2* correlated with the presence of a functional androgen receptor in the cell. These data support the finding that *STAMP2* is expressed in a tissue-restricted manner and that it is an androgen target gene in androgen responsive LNCaP cells.

Example 4. Intracellular localization of STAMP2.

To gain insight into the cellular localization pattern of STAMP2, we labeled it with the Green Fluorescent Protein (GFP) to generate GFP-STAMP2. Such use of GFP fusion proteins is frequently used to assess intracellular localization and dynamics of proteins (Chalfie et al., Science, 263:802-805, 1994; for a review, see Tsien, *Annu. Rev. Biochem.* 67:509-544, 1998). COS-1 cells were transiently transfected with GFP-STAMP2, fixed and processed for confocal microscopy. A representative single optical section of a COS-1 cell is shown in Figure 5A. GFP-STAMP2 displays a strong juxtanuclear distribution that is typical of the Golgi complex. Additionally, significant GFP-STAMP2 distribution

was observed in the cell periphery and in vesicular or tubular-shaped bright spots throughout the cytoplasm suggesting that STAMP2 localizes to plasma membrane (PM) and to vesiculotubular structures (VTS) in the cytosol (Figure 5A). At higher exposures, GFP-STAMP2 distribution can also be seen as a light reticular pattern in the cytosol, reminiscent of the endoplasmic reticulum (Figure 5A, right panel).

In order to directly assess whether GFP-STAMP2 is associated with the Golgi complex, we compared the intracellular distribution of GFP-STAMP2 with those of three well characterized Golgi markers, the coat protein β-COP (Pepperkok et al., *Cell* 74:71-82, 1993), mid-Golgi marker giantin (Linstedt et al., *Mol. Biol. Cell* 4:679-693, 1993), and trans-Golgi network (TGN) marker TGN-46 (Ponnambalam et al., *Curr. Biol.* 6:1076-1078, 1996). GFP-STAMP2 was transfected into COS-1 cells, which were then fixed, labeled with the appropriate primary and secondary antibodies, and then single optical sections were acquired by laser scanning confocal microscopy. As shown in Figure 5B, there was significant overlap of GFP-STAMP2 juxtanuclear distribution with Golgi and TGN markers, but there were also some differences. This suggests that STAMP2, at least in part, is differentially localized within the Golgi complex. Expression of STAMP2 in the Golgi suggests that six-transmembrane proteins may have unique functions in this organelle compared with the other Golgi resident proteins identified so far. Photobleaching experiments provided evidence that GFP-STAMP2 is rapidly exchanged between different parts of the Golgi with kinetics consitent with its role as a transmembrane protein.

Example 5. GFP-STAMP2 is a highly mobile protein and is associated with vesiculotubular structures in the cytosol.

To gain insight into the possible function of STAMP2, the kinetic properties of GFP-STAMP2 distribution and trafficking were studied in living cells by time-lapse confocal microscopy. COS-1 cells were transfected with GFP-STAMP2. 18 hours after transfection, images were obtained from live cells at 3 second intervals at 37°C.

There was rapid trafficking of GFP-STAMP2 in the cytosol in the form of predominantly vesiculotubular structures (VTS; Figure 6). Some of the VTSs followed straight or curvilinear paths, some moved in a stop-and-go fashion, and some showed saltatory movements. The movement of one such VTS from cytoplasm to cell periphery is shown in consecutive images in Figure 6. These data suggest that STAMP2 is associated with the secretory pathway.

To probe whether GFP-STAMP2 was associated with the endocytic pathway, we compared the intracellular distribution of GFP-STAMP2 with that of the early endosome protein EEA1 (Stenmark et al., *J. Biol. Chem.* 271, 24048-24054, 1996). GFP-STAMP2 was transfected into COS-1 cells, which were then fixed, immunostained with EEA1 antibodies, and observed by laser scanning confocal microscopy. As shown in Figure 7, EEA1 had similar intracellular distribution in both transfected and untransfected cells. GFP-STAMP2 significantly colocalized with EEA1 both in the cell periphery and also in the perinuclear area (Figure 7) suggesting that STAMP2 is associated with early endosomes and the endocytic pathway. The significant STAMP2 distribution in the plasma membrane and the movement of STAMP2 within transport vesicles to and from the plasma membrane in live cell imaging studies suggest that STAMP2 may be involved in the secretory and endocytic pathways.

In addition to the localization of GFP-STAMP2 in the Golgi, TGN, and the plasma membrane, a fraction of the GFP-STAMP2 protein can also be detected in the endoplasmic reticulum (ER). ER is the central organelle that is necessary for proper folding and delivery of proteins. Proteins destined for secretion, the plasma membrane or the cell surface, are translocated from the cytoplasm into the ER for further delivery of these proteins to their site of action. Since proteins are translocated into the ER in an unfolded state, it is the primary function of this organelle to modify and fold the translocated proteins to acquire their biologically active conformation (Haigh et al., Protein sorting at the membrane of the endoplasmic reticulum. In *Protein Targeting, Transport, and Translocation*, Dalbey R.E. and von Heine G. (eds.), Academic Press, London, UK, pp.74-106, 2002). We do not know the significance of the presence of STAMP2 in the ER, or whether it has a functional role in this organelle. However, since the majority of STAMP2 expression is in the Golgi, TGN, early endosomes and the plasma membrane, it is possible that GFP-STAMP2 is unfolded in the ER and is kept there for further processing. Alternatively, there may be a functional role of STAMP2 in the ER.

Example 6. Analysis of STAMP2 expression in normal vs. adenocarcinoma of prostate.

Based on the tissue specific expression of STAMP2 in prostate tissues and the androgen regulation of *STAMP2* in androgen receptor positive prostate cancer cells, we studied its expression in normal prostate epithelial cells compared with adenocarcinoma of the prostate. To that end, Laser Capture Microdissection (LCM) (Bonner et al., *Science* 278:1481-1483, 1997) was used on human radical prostatectomy specimens that were snap frozen immediately upon resection. Normal epithelial cells and cancer cells from the same specimen were collected

(matched normal/tumor pair). Total RNA was extracted and cDNA was made and used in a quantitative RT-PCR assay with *STAMP2*-specific primers. The results of this experiment representing normal/tumor pairs from 26 independent patient samples are presented in Figure 8. STAMP2 expression was significantly higher in tumors compared with normal galnds (p=0.0002). LNCaP cells treted with R1881 for 24 hours were used as a positive control. The results of these experiments showed significant *STAMP2* amplification in the control LNCaP cells treated with R1881. In contrast, none of the normal cell pools from prostatectomy specimens showed any *STAMP2* expression. Interestingly, 5 of the 26 samples (#15, 21, 22, 24, 26) of cancer cell pools (~20%) in the prostate specimens showed very high *STAMP2* mRNA expression reaching up to 30-fold higher than that observed in R1881-treated LNCaP cells. These data indicate that *STAMP2* may have a role in the genesis of, and be a marker for, at least a subset of prostate cancers.

Example 7. Androgen regulation of STAMP2 protein expression.

Since STAMP2 RNA was shown to be androgen-regulated (Example 3), we sought to determine if STAMP2 protein was also androgen-regulated. For these experiments LNCaP cells were either left untreated or treated with R1881 (10^{-8} M) for the indicated times. The whole cell extracts (from a 10 cm dish) were prepared by resuspending the cells in 200 µl of lysis buffer (20 mM HEPES (pH 7.4), 300 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.1% Triton X-100, 0.5 mM DTT, 20 mM β-glycerophosphate, 0.1 mM $NaVO_3$, 0.5 mM PMSF, 1 mM leupeptin, 5 µg/ml aprotinin). The suspension was rotated at 4° C for 2 hours, followed by centrifugation at 15,000× g for 30 minutes. The supernatant was collected and stored at 80 C. The protein concentrations were determined by the Bio-Rad Protein Assay. Protein extracts (100 µg) were electrophoresed on 10%

SDS-PAGE gel. The proteins were then transferred to a PVDF membrane (BIO-RAD), which was blocked with 5% milk for 2 hours, followed by incubation with the primary antibody at 4 C overnight. The ECL kit (Amersham Pharmacia) was used for detection according to the manufacturer's recommendations. 1/1000 dilution of the antiserum was used as the primary antibody. The results of this experiment demonstrate the androgen responsiveness of the STAMP2 protein (Figure 9).

Example 8. Ectopic expression of STAMP2 increases colony formation and cellular proliferation.

The effects of ectopic expression of STAMP2 on cell growth were analyzed using DU145 cells. Colony formation assays were performed as described below in the Materials and Methods. Cells were either transfected with en empty expression plasmid pCDNA3 or one that encodes full length STAMP2 cDNA. 24 hours after transfection, cells were trypsinized, selected for plasmid integration in G418, and cultured for two weeks. Colony formation was detected by staining with crystal violet (0.1%) and measuring the area covered on each plate by the colonies using an imaging system (Syngene). The results shown in Figures 10A and 10B demonstrate that STAMP2 expression increases colony formation in DU145 cells. Similar results were found in PC3 cells which do not express STAMP2. These results suggest a role for STAMP2 expression or overexpression in the induction of cell growth and proliferation. Results represent 3-5 experiments done at least in duplicate.

The effect of STAMP2 on cell growth and proliferation was also measured in DU145 cells and COS7 cells. For these experiments cells were either transfected with an empty expression plasmid plus a GFP expression vector (control), or the GFP expression vector together with an expression vector that

encodes full length STAMP2 cDNA in a 1:5 ratio, to ensure that the great majority of the cells that have the STAMP2 cDNA also have GFP. At indicated time points, cells were trypsinized, fixed, and then counted for the GFP content by a FACS machine. The numbers of GFP positive cells were obtained and presented as the ratio between STAMP2 transfected compared with controls. The results shown in Figures 11 and 12 demonstrate that ectopic expression of STAMP2 increases cell proliferation in DU145 cells and COS7 cells. Results presented are from at least two experiments done in duplicate.

Material and Methods

The following materials and methods were used to conduct the experiments described above.

Cell culture. All prostate cancer cell lines were routinely maintained and treated as previously described (Korkmaz et al., *DNA Cell Biol.* 19:499-506, 2000). Briefly, the cells were routinely maintained in RPMI 1640 medium (Gibco-BRL), supplemented with 10% fetal calf serum (FCS), penicillin/streptomycin (5 mg/ml), and L-glutamine (200 mM). For androgen induction and RNA preparation, cells were grown for 48 hours in RPMI 1640 containing 2% FCS that was charcoal-treated (CT) to remove steroids, followed by an additional 24 hours in RPMI 1640 containing 0.5% CT-FCS. The synthetic androgen R1881 (10^{-8} M) (Dupont-NEN) was then added and cells were collected at indicated time points. Total RNA was prepared by the single step guanidine thiocyanate procedure and used in Northern analysis. All other cell lines were cultured in DMEM supplemented with 10% fetal calf serum (FCS), penicillin/streptomycin (5 mg/ml), and L-glutamine (200 mM).

Cloning and plasmid construction. Several partial cDN Aclones as well as genomic clones were identified in a GenBank screen for sequences similar to STAMP1 (Korkmaz et al., *J. Biol. Chem.* 277:36689-36696, 2002). We termed this novel gene *STAMP2*. These *STAMP2* clones were then used to computationally construct a full-length cDNA. Primers were designed on the basis of this sequence and used in PCR to amplify the full-length STAMP2 cDNA from an LNCaP SMART (Clontech) cDNA library. The amplified cDNA product was cloned into pCRII-TOPO, or pcDNA4-HisMax (Invitrogen, CA) and sequenced on both strands. In addition, the resulting cDNA sequence was aligned with the gene sequence (BAC AC003991) to determine gene structure and sequence accuracy.

The full-length *STAMP2* ORF from pCRII-TOPO-STAMP2 was fused in frame to the C-terminus of green fluorescent protein (GFP) using the vector pEGFP-C1 (Clontech) to generate GFP-STAMP2.

DNA sequence analysis. For DNA sequencing, BigDye kit (Perkin Elmer), and automated dye termination system services of GATC, Germany, was used. The DNA sequences were analyzed by standard algorithms accessible at various web sites, including NCBI-BLAST, Clustal 1.8, and Gendoc 2.6.

Protein sequence analysis. Primary sequence analysis for STAMP1 was performed by BLAST at http://www.ncbi.nlm.nih.gov/BLAST/. Secondary protein structure predictions were performed by using the web tools SMART, at http://smart.embl-heidelberg.de/, SOSUI at http://sosui.proteome.bio.tuat.ac.jp/sosuiframe0E.html, and PSORT at http://psort.nibb.ac.jp/

Northern analysis. Total RNA was prepared by the single step guanidine thiocyanate procedure and used in Northern analysis (Ausubel et al. Current Protocols in Molecular Biology, John Wiley and Sons, New York, 1997). 15 μg of total RNA was used per lane. Probes were generated by random priming and had a specific activity of $>3x10^8$ dpm/μg. A full-length cDNA (1-2263 bp) of *STAMP2* (GenBank Accession number # AF423422) was used as probe. Bands were visualized and quantitated by phosphorimager analysis (Molecular Dynamics).

Confocal microscopy and live cell imaging. COS-1 cells were transfected by Fugene (Roche). Cells were grown either on cover slips placed in 6-well tissue culture plates for indirect immunofluorescence microscopy or on Lab-Tek Chambered Coverglass (Nalgene Nunc International) for live-cell microscopy. For immunoflourescence, transiently transfected cells were observed 18 hours after transfection by Leica TCS-SP laser scanning confocal microscope using 488-nm Argon laser line. All live-cell experiments were done on a Zeiss 510 laser scanning confocal microscope using a 100x/1.3 N.A. oil immersion objective and 40 mW argon laser at 37 C. Imaging was carried out in the Fluorescence Imaging Facility, Laboratory of Receptor Biology and Gene Expression, National Cancer Institute.

Indirect immunofluorescence. Indirect immunofluorescence was carried out as previously described (Misteli et al., *Mol. Cell* 3:697-705, 1999). The following antibodies were used: anti-β-coat protein (β -COP) (Affinity Bioreagents), anti-giantin (Covance), anti-TGN46 (Serotec), and anti-EEA1 (BD Transduction

Labs). Texas Red-conjugated secondary antibodies specific for mouse and rabbit were purchased from Jackson Immunoresearch Laboratories.

Prostate tissue preparation and microdissection. Radical prostatectomy specimens were obtained at Aker University Hospital and snap frozen upon resection. Sections were obtained and microdissected by a pathologist using Laser Capture Microdissection (LCM) as previously described (Bonner et al., *Science* 278:1481-1483, 1997).

Quantitative RT-PCR. Total RNA was extracted from the LCM samples using Absolutely RNA™ Microprep Kit (Stratagene), including DNAse treatment. RNA was used for first strand cDNA synthesis with the Superscript II system (Invitrogen). *STAMP2* expression level was determined using the Light Cycler Instrument (Roche, Mannheim, Germany), with the Light Cycler-FastStart DNA Master SYBR Green I Kit (Roche). The primers used were Forward: 5'-TGC AAG TCG GCA GGT GTT TG-3', and Reverse: 5'-GCA AAG CAT CCA GTG GTC AA-3'. A standard curve made from serial dilutions of cDNA was used to calculate the relative amount of *STAMP2* in each sample. These values were then normalized to the relative amount of the internal standard ATP6 Synthase (ATP-6) in the same samples, calculated from a standard curve established in the same way. Primers used for ATP-6 amplification were Forward: 5'-CAG TGA TTA TAG GCT TTC GCT CTA A-3', and Reverse: 5'-CAG GGC TAT TGG TTG AAT GAG TA-3'. The experiment was repeated twice with similar results.

Colony formation assay. Cells were either transfected with en empty expression plasmid pCDNA3 or one that encodes full length STAMP2 cDNA. 24 hours after transfection, cells were trypsinized and seeded on new plates (10000 cells/100

mm^2 dish). Selection antibiotic (G418, Sigma) was added and cells were cultured for two weeks. Cells were then fixed with cold methanol at -20 C for 30 minutes. The colonies formed were stained with crystal violet (0.1%) and the area covered on each plate by the colonies were measured using an imaging system (Syngene). Results represent 3-5 experiments done at least in duplicate.

Effect of STAMP2 on cell growth. Cells were either transfected with an empty expression plasmid plus a GFP expression vector (control), or the GFP expression vector together with an expression vector that encodes full length STAMP2 cDNA in 1 to 5 ratio, thus insuring that the great majority of the cells that have the STAMP2 cDNA also have GFP. At indicated time points, cells were trypsinized, fixed with cold methanol at -20 C for 30 minutes, and then counted for the GFP content by a FACS machine. The numbers of GFP positive cells were obtained and presented as the ratio between STAMP2 transfected compared with controls. Results presented are from at least two experiments done in duplicate.

Other Embodiments

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be

applied to the essential features hereinbefore set forth, and follow in the scope of the appended claims.

What is claimed is:

CLAIMS

1. A method of diagnosing a subject as having, or having a propensity to develop, a disorder of the prostate or testis, said method comprising measuring the level of STAMP2 polypeptide in a sample from said subject.

2. The method of claim 1, wherein said measuring is done using an immunological assay.

3. The method of claim 1, wherein said immunological assay is an ELISA.

4. The method of claim 1, further comprising comparing said level to a reference.

5. The method of claim 4, wherein said reference is a normal reference and an increase in the level of STAMP2 polypeptide in said sample from said subject relative to said reference, is a diagnostic indicator of a disorder of the prostate or testis or a propensity to develop a disorder of the prostate or testis.

6. A method of diagnosing a subject as having, or having a propensity to develop, a disorder of the prostate or testis, said method comprising measuring the level of *STAMP2* polynucleotide in a sample from said subject.

7. The method of claim 6, further comprising comparing said level to a reference.

8. The method of claim 7, wherein said reference is a normal reference and an increase in the level of STAMP2 polynucleotide in said sample from said subject relative to said reference, is a diagnostic indicator of a disorder of the prostate or testis or a propensity to develop a a disorder of the prostate or testis.

9. The method of claim 1 or 6, wherein said measuring of levels is done on two or more occasions and a change in said levels between measurements is a diagnostic indicator of a disorder of the prostate or testis.

10. The method of claim 9, wherein said change is an increase in said levels.

11. A method of diagnosing a subject as having, or having a propensity to develop, a disorder of the prostate or testis, said method comprising determining the nucleic acid sequence of a *STAMP2* gene in a sample from a subject and comparing it to a reference sequence, wherein an alteration in the subject's nucleic acid sequence that is an alteration that changes the expression level or biological activity of the STAMP2 gene product in said subject diagnoses the subject with a disorder of the prostate, or a propensity to develop a disorder of the prostate or testis.

12. The method of claim 1, 6, or 11, wherein said sample is selected from the group consisting of blood, serum, urine, and semen.

13. The method of claim 1, 6, or 11, wherein said sample is a cell or a tissue.

14. The method of claim 13, wherein said cell or tissue sample is derived from a prostate or testis.

15. The method of claim 1, 6, or 11, wherein said subject is selected from the group consisting of a human, a cow, a horse, a sheep, a pig, a goat, a dog, and a cat.

16. A kit for the analysis of a *STAMP2* nucleic acid molecule, said kit comprising a *STAMP2* nucleic acid molecule probe at least 80% identical to *STAMP2* cDNA or a fragment thereof, wherein said probe hybridizes under high stringecy conditions to the sequence set forth in SEQ ID NO: 1 or the complementary sequences thereof.

17. The kit of claim 16, wherein said probe comprises a substantially pure polynucleotide comprising a sequence encoding the STAMP2 amino acid sequence set forth in SEQ ID NO: 2 or fragment thereof.

18. A kit for the diagnosis of a disorder of the prostate or testis in a subject, said kit comprising a means of detecting a STAMP2 polypeptide or any fragment thereof.

19. The kit of claim 18, wherein said means of detecting is selected from the group consisting of an immunological assay, an enzymatic assay, and a colorimetric assay.

20. The kit of claim 18, wherein said kit comprises an antibody that specifically binds a STAMP2 polypeptide in a sample taken from said subject.

21. A substantially pure polynucleotide comprising a sequence at least 80% identical to the *STAMP2* nucleic acid sequence set forth in SEQ ID NO: 1, or a fragment thereof.

22. The substantially pure polynucleotide of claim 21 comprising the *STAMP2* nucleic acid sequence set forth in SEQ ID NO: 1.

23. The substantially pure polynucleotide of claim 21, wherein said polynucleotide consists of the nucleic acid sequence of SEQ ID NO: 1.

24. A substantially pure polynucleotide comprising a sequence encoding an amino acid sequence at least 80% identical to the STAMP2 amino acid sequence set forth in SEQ ID NO: 2, or fragment thereof.

25. A substantially pure polynucleotide comprising a sequence encoding the STAMP2 amino acid sequence set forth in SEQ ID NO: 2.

26. The substantially pure polynucleotide of claim 25, consisting of a sequence encoding the STAMP2 amino acid sequence set forth in SEQ ID NO: 2.

27. A sustantially pure polynucleotide comprising a sequence at least 80% identical to nucleotides 107 to 167 of SEQ ID NO: 1.

28. A substantially pure polynucleotide comprising a sequence at least 80% identical to nucleotides 1306 to 1360 of SEQ ID NO: 1.

29. A substantially pure polynucleotide probe comprising a sequence that hybridizes at high stringency to the *STAMP2* nucleic acid sequence set forth SEQ ID NO: 1, or a fragment thereof.

30. A substantially pure polynucleotide probe comprising a sequence that hybridizes at high stringency to a polynucleotide encoding the STAMP2 amino acid sequence set forth SEQ ID NO: 2, or a fragment thereof.

31. A substantially pure polypeptide comprising a sequence at least 80% identical to the amino acid sequence of SEQ ID NO: 2.

32. A substantially pure polypeptide comprising a sequence at least 80% identical to amino acids 1 to 20 of SEQ ID NO: 2.

33. A substantially pure polypeptide comprising a sequence at least 80% identical to amino acids 70 to 82 of SEQ ID NO: 2.

34. A substantially pure polypeptide comprising a sequence at least 80% identical to amino acids 87 to 97 of SEQ ID NO: 2.

35. A substantially pure polypeptide comprising a sequence at least 80% identical to amino acids 330 to 347 of SEQ ID NO: 2.

36. A substantially pure polypeptide comprising a sequence at least 80% identical to amino acids 400 to 428 of SEQ ID NO: 2.

37. A substantially pure polypeptide comprising a sequence at least 80% identical to amino acids 445 to 459 of SEQ ID NO: 2.

38. An antibody that specifically binds a STAMP2 polypeptide comprising the sequence of SEQ ID NO: 2.

39. An antibody that specifically binds a polypeptide comprising the sequence of amino acids 1 to 20 of SEQ ID NO: 2.

40. An antibody that specifically binds a polypeptide comprising the sequence of amino acids 70 to 82 of SEQ ID NO: 2.

41. An antibody that specifically binds a polypeptide comprising the sequence of amino acids 87 to 97 of SEQ ID NO: 2.

42. An antibody that specifically binds a polypeptide comprising the sequence of amino acids 330 to 347 of SEQ ID NO: 2.

43. An antibody that specifically binds a polypeptide comprising the sequence of amino acids 400 to 428 of SEQ ID NO: 2.

44. An antibody that specifically binds a polypeptide comprising the sequence of amino acids 445 to 459 of SEQ ID NO: 2.

45. A method of treating a subject having a disorder of the prostate or testis, said method comprising administering to said subject, said method comprising the step of administering to said subject a compound capable of reducing STAMP2 expression or biological activity, wherein said administering is sufficient to treat or prevent said disorder of the prostate or testis in said subject.

46. The method of claim 45, said compound comprising an antisense nucleobase oligomer at least 80% complementary to at least a portion of SEQ ID NO: 1, wherein said administering is sufficient to treat or prevent said disorder of the prostate or testis in said subject.

47. The method of claim 46, wherein said antisense nucleobase oligomer is 8 to 30 nucleotides in length.

48. The method of claim 45, said compound comprising a double stranded RNA at least 80% complementary to at least a portion of SEQ ID NO: 1, wherein said administering is sufficient to treat or prevent a disorder of the prostate or testis in said subject.

49. The method of claim 48, wherein said double stranded RNA is processed into small interfering RNAs (siRNAs) 19 to 25 nucleotides in length.

50. The method of claim 45, said compound comprising a purified antibody or antigen-binding fragment that specifically binds to STAMP2.

51. A method of identifying a compound that ameliorates a disorder of the prostate or testis, said method comprising contacting a cell that expresses a *STAMP2* nucleic acid molecule with a candidate compound, and comparing the level of expression of said *STAMP2* nucleic acid molecule in said cell contacted by said candidate compound with the level of expression in a control cell not contacted by said candidate compound, wherein an alteration in expression of said *STAMP2*nucleic acid molecule identifies said candidate compound as a compound that ameliorates a disorder of the prostate or testis

52. The method of claim 51, wherein said alteration is a decrease in the level of said *STAMP2* nucleic acid molecule.

53. A method of identifying a compound that ameliorates a disorder of the prostate or testis, said method comprising contacting a cell that expresses a STAMP2 polypeptide with a candidate compound, and comparing the level of expression of said STAMP2 polypeptide in said cell contacted by said candidate compound with the level of polypeptide expression in a control cell not contacted by said candidate compound, wherein an alteration in the expression of said STAMP2 polypeptide identifies said candidate compound as a compound that ameliorates a disorder of the prostate or testis.

54. The method of claim 53, wherein said alteration in expression is assayed using an immunological assay, an enzymatic assay, or an immunoassay.

55. The method of claim 53, wherein said alteration in expression is a decrease in the level of STAMP2 polypeptide.

56. A method of identifying a compound that ameliorates a disorder of the prostate or testis, the method comprising contacting a cell that expresses a STAMP2 polypeptide with a candidate compound, and comparing the biological activity of said STAMP 2polypeptide in said cell contacted by said candidate compound with the level of biological activity in a control cell not contacted by said candidate compound, wherein an alteration in the biological activity of said STAMP2 polypeptide identifies said candidate compound as a compound that ameliorates a disorder of the prostate or testis.

57. A vector comprising a nucleic acid molecule positioned for expression, wherein said nucleic acid molecule encodes a nucleic acid molecule having at least one strand that is complementary to at least a portion of SEQ ID NO: 1.

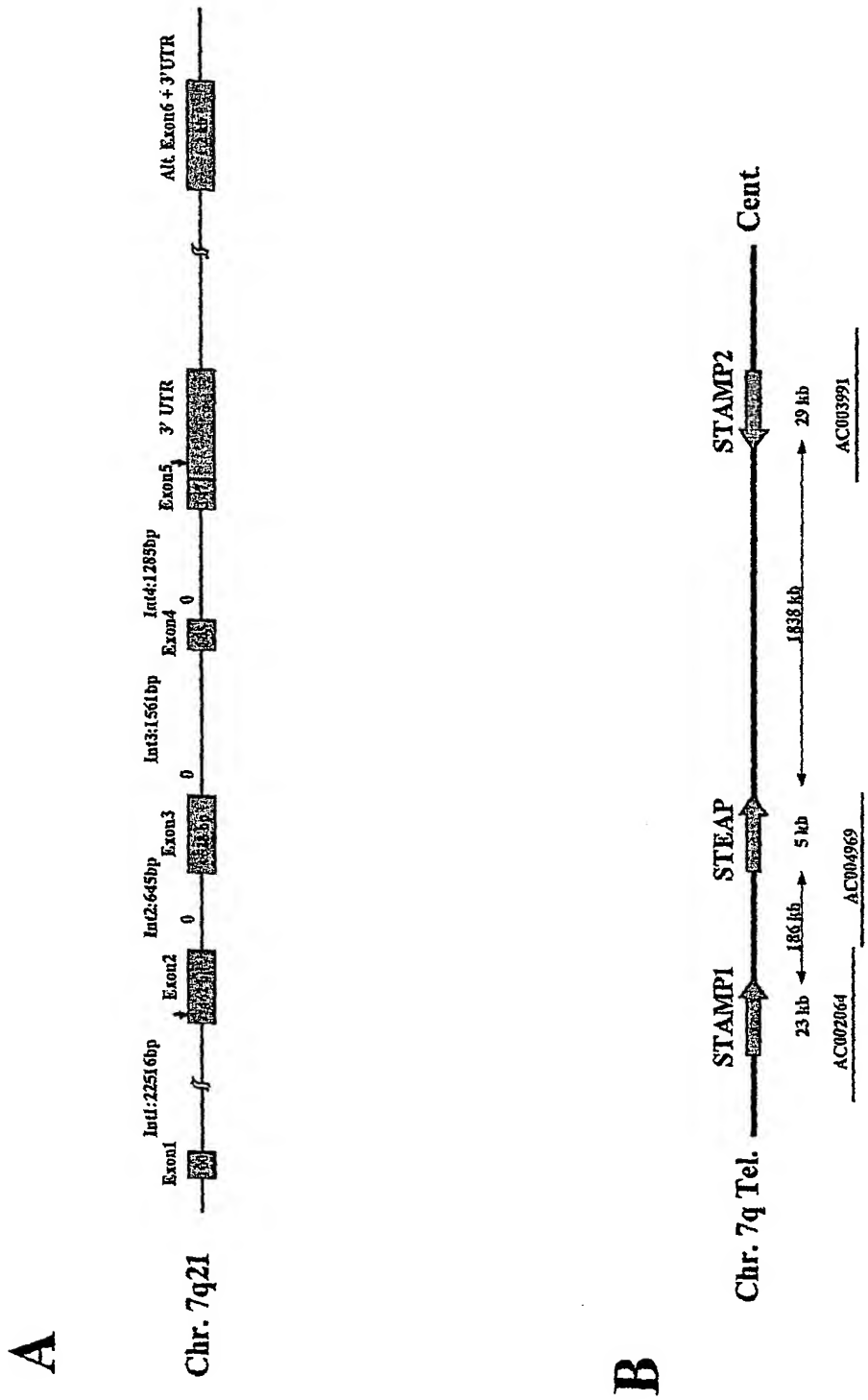

A
Chr. 7q21
Exon1
Int1:22516bp
Exon2
Int2:645bp
Exon3
Int3:1561bp
Exon4
Int4:1285bp
Exon5
3' UTR
Alt. Exon6 + 3'UTR
B
Chr. 7q Tel.
STAMP1
STEAP
STAMP2
Cent.
23 kb
186 kb
5 kb
1838 kb
29 kb
AC002064
AC004969
AC003991

Figure 1

```
   1 gCTGAGCTGCAGGCGCGGCGAAACTTCCCTCTACCCGCCCGGCCCGCGGCGCGCACCGTTG
   1                                                       M  E  K  T  C
  62 GCGCTGGACGCTTCCTCCTTGGAAGCGCCTCTCCCTCAGTCAGTTATGGAGAAAACTTGT
   6  I  D  A  L  P  L  T  M  N  S  S  E  K  Q  E  T  V  C  I  F
 122 ATAGATGCACTTCCTCTTACTATGAATTCTTCAGAAAAGCAAGAGACTGTATGTATTTTT
  26  G  T  G  D  F  G  R  S  L  G  L  K  M  L  Q  C  G  Y  S  V
 182 GGAACTGGTGATTTTGGAAGATCACTGGGATTGAAAATGCTCCAGTGTGGTTATTCTGTT
  46  V  F  G  S  R  N  P  Q  K  T  T  L  L  P  S  G  A  E  V  L
 242 GTTTTTGGAAGTCGAAACCCCCAGAAGACCACCCTACTGCCCAGTGGTGCAGAAGTCTTG
  66  S  Y  S  E  A  A  K  K  S  G  I  I  I  I  A  I  H  R  E  H
 302 AGCTATTCAGAAGCAGCCAAGAAGTCTGGCATCATAATCATAGCAATCCACAGAGAGCAT
  86  Y  D  F  L  T  E  L  T  E  V  L  N  G  K  I  L  V  D  I  S
 362 TATGATTTTCTCACAGAATTAACTGAGGTTCTCAATGGAAAAATATTGGTAGACATCAGC
 106  N  N  L  K  I  N  Q  Y  P  E  S  N  A  E  Y  L  A  H  L  V
 422 AACAACCTCAAAATCAATCAATATCCAGAATCTAATGCAGAGTACCTTGCTCATTTGGTG
 126  P  G  A  H  V  V  K  A  F  N  T  I  S  A  W  A  L  Q  S  G
 482 CCAGGAGCCCACGTGGTAAAAGCATTTAACACCATCTCAGCCTGGGCTCTCCAGTCAGGA
 146  A  L  D  A  S  R  Q  V  F  V  C  G  N  D  S  K  A  K  Q  R
 542 GCACTGGATGCAAGTCGGCAGGTGTTTGTGTGTGGAAATGACAGCAAAGCCAAGCAAAGA
 166  V  M  D  I  V  R  N  L  G  L  T  P  M  D  Q  G  S  L  M  A
 602 GTGATGGATATTGTTCGTAATCTTGGACTTACTCCAATGGATCAAGGATCACTCATGGCA
 186  A  K  E  I  E  K  Y  P  L  Q  L  F  P  M  W  R  F  P  F  Y
 662 GCCAAAGAAATTGAAAAGTACCCCCTGCAGCTATTTCCAATGTGGAGGTTCCCCTTCTAT
 206  L  S  A  V  L  C  V  F  L  F  F  Y  C  V  I  R  D  V  I  Y
 722 TTGTCTGCTGTGCTGTGTGTCTTCTTGTTTTTCTATTGTGTTATAAGAGACGTAATCTAC
 226  P  Y  V  Y  E  K  K  D  N  T  F  R  M  A  I  S  I  P  N  R
 782 CCTTATGTTTATGAAAAGAAAGATAATACATTTCGTATGGCTATTTCCATTCCAAATCGT
 246  I  F  P  I  T  A  L  T  L  L  A  L  V  Y  L  P  G  V  I  A
 842 ATCTTTCCAATAACAGCACTTACACTGCTTGCTTTGGTTTACCTCCCTGGTGTTATTGCT
 266  A  I  L  Q  L  Y  R  G  T  K  Y  R  R  F  P  D  W  L  D  H
 902 GCCATTCTACAACTGTACCGAGGCACAAAATACCGTCGATTCCCAGACTGGCTTGACCAC
 286  W  M  L  C  R  K  Q  L  G  L  V  A  L  G  F  A  F  L  H  V
 962 TGGATGCTTTGCCGAAAGCAGCTTGGCTTGGTAGCTCTGGGATTTGCCTTCCTTCATGTC
 306  L  Y  T  L  V  I  P  I  R  Y  Y  V  R  W  R  L  G  N  L  T
1022 CTCTACACACTTGTGATTCCTATTCGATATTATGTACGATGGAGATTGGGAAACTTAACC
 326  V  T  Q  A  I  L  K  K  E  N  P  F  S  T  S  S  A  W  L  S
1082 GTTACCCAGGCAATACTCAAGAAGGAGAATCCATTTAGCACCTCCTCAGCCTGGCTCAGT
 346  D  S  Y  V  A  L  G  I  L  G  F  F  L  F  V  L  L  G  I  T
1142 GATTCATATGTGGCTTTGGGAATACTTGGGTTTTTTCTGTTTGTACTCTTGGGAATCACT
 366  S  L  P  S  V  S  N  A  V  N  W  R  E  F  R  F  V  Q  S  K
1202 TCTTTGCCATCTGTTAGCAATGCAGTCAACTGGAGAGAGTTCCGATTTGTCCAGTCCAAA
 386  L  G  Y  L  T  L  I  L  C  T  A  H  T  L  V  Y  G  G  K  R
1262 CTGGGTTATTTGACCCTGATCTTGTGTACAGCCCACACCCTGGTGTACGGTGGGAAGAGA
 406  F  L  S  P  S  N  L  R  W  Y  L  P  A  A  Y  V  L  G  L  I
1322 TTCCTCAGCCCTTCAAATCTCAGATGGTATCTTCCTGCAGCCTACGTGTTAGGGCTTATC
 426  I  P  C  T  V  L  V  I  K  F  V  L  I  M  P  C  V  D  N  T
1382 ATTCCTTGCACTGTGCTGGTGATCAAGTTTGTCCTAATCATGCCATGTGTAGACAACACC
 446  L  T  R  I  R  Q  G  W  E  R  N  S  K  H  -
1442 CTTACAAGGATCCGCCAGGGCTGGGAAAGGAACTCAAAACACTAG
```

Figure 2

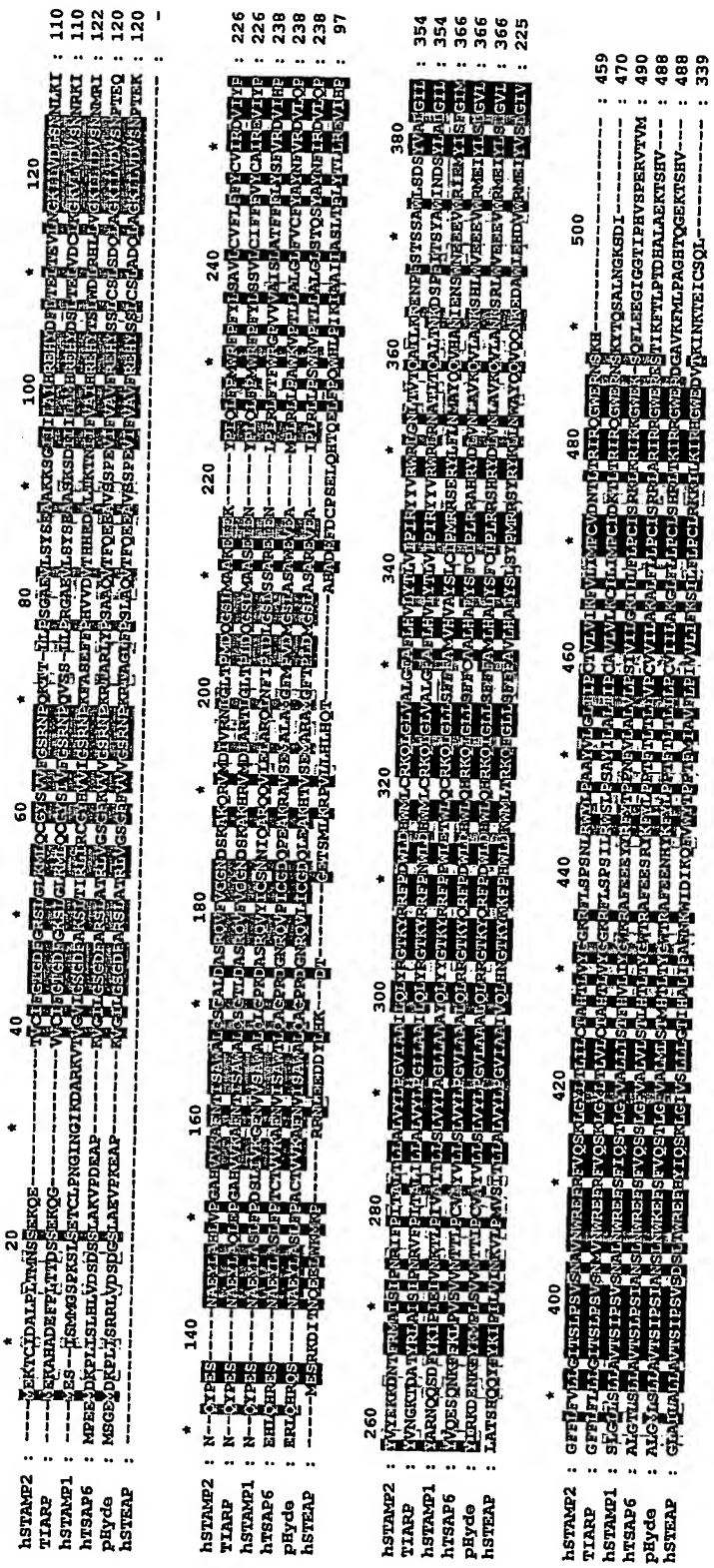


Figure 3

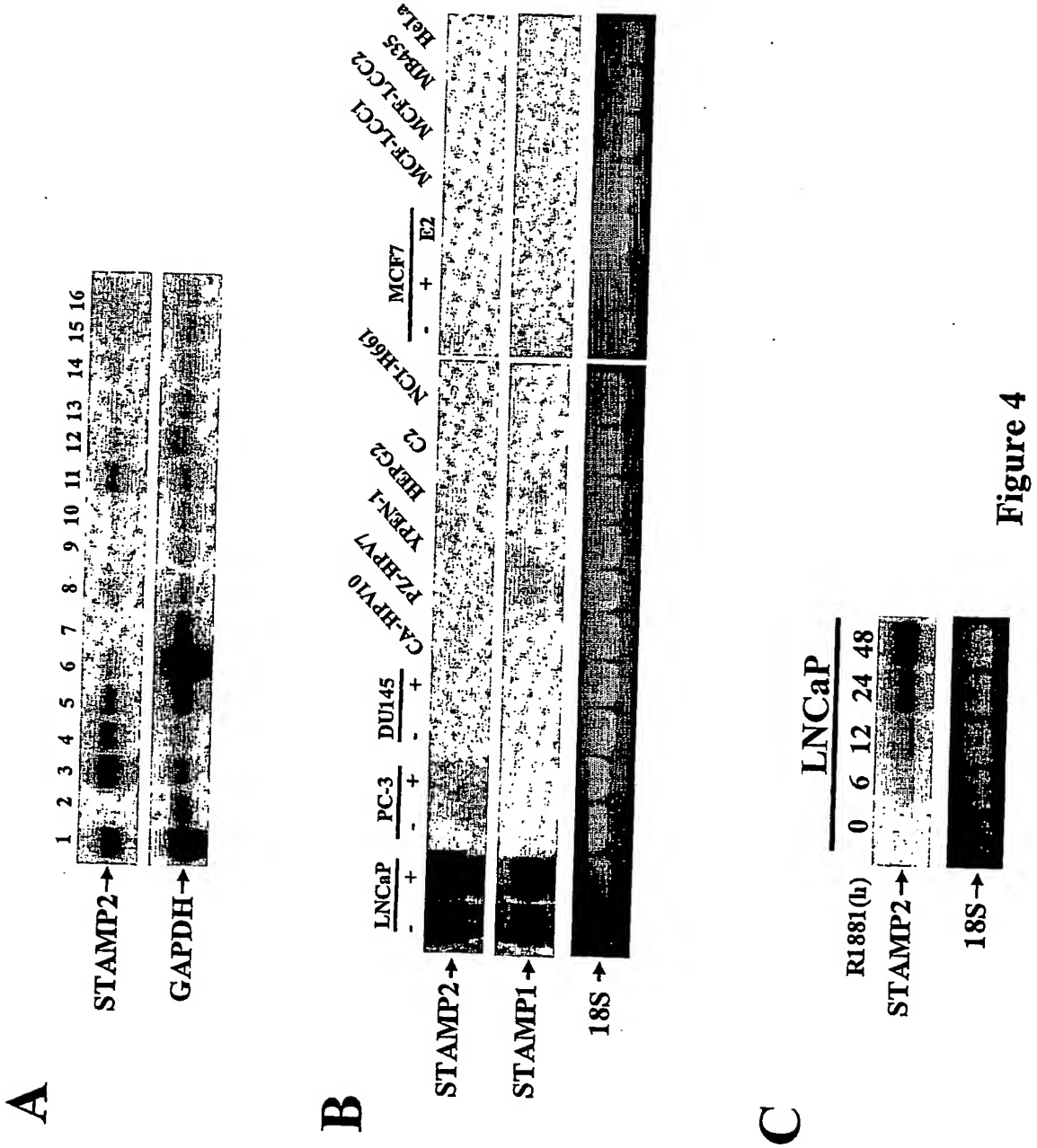

A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
STAMP2→
GAPDH→
B
LNCaP - +
PC-3 - +
DU145 - +
CA-HPV10
PZ-HPV7
YPEN-1
HEPG2
C2
NCI-H661
MCF7 - E2
MCF-LCC1
MCF-LCC2
MBA35
HeLa
STAMP2→
STAMP1→
18S→
C
LNCaP
R1881(h) 0 6 12 24 48
STAMP2→
18S→

Figure 4

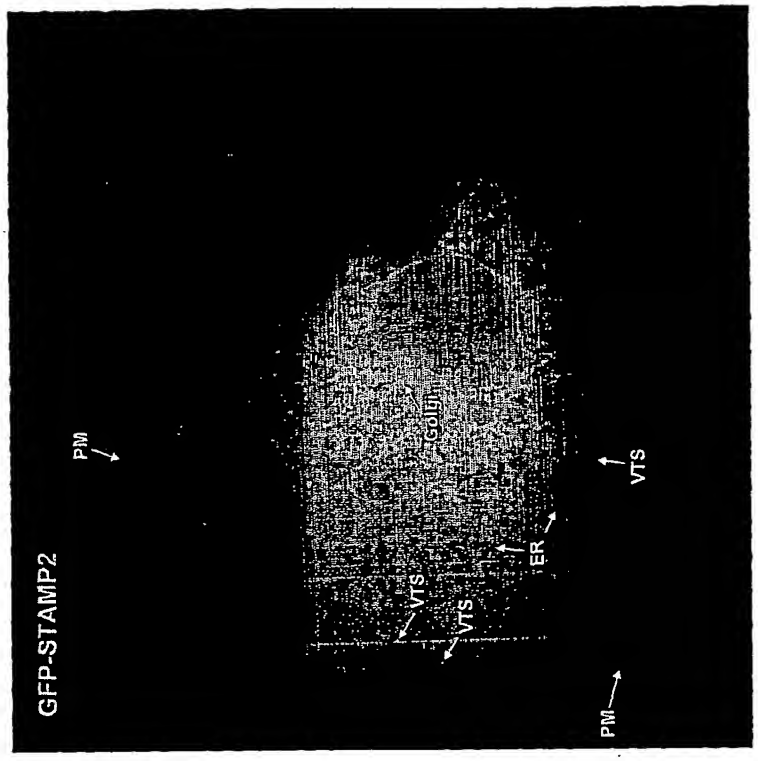


Figure 5A

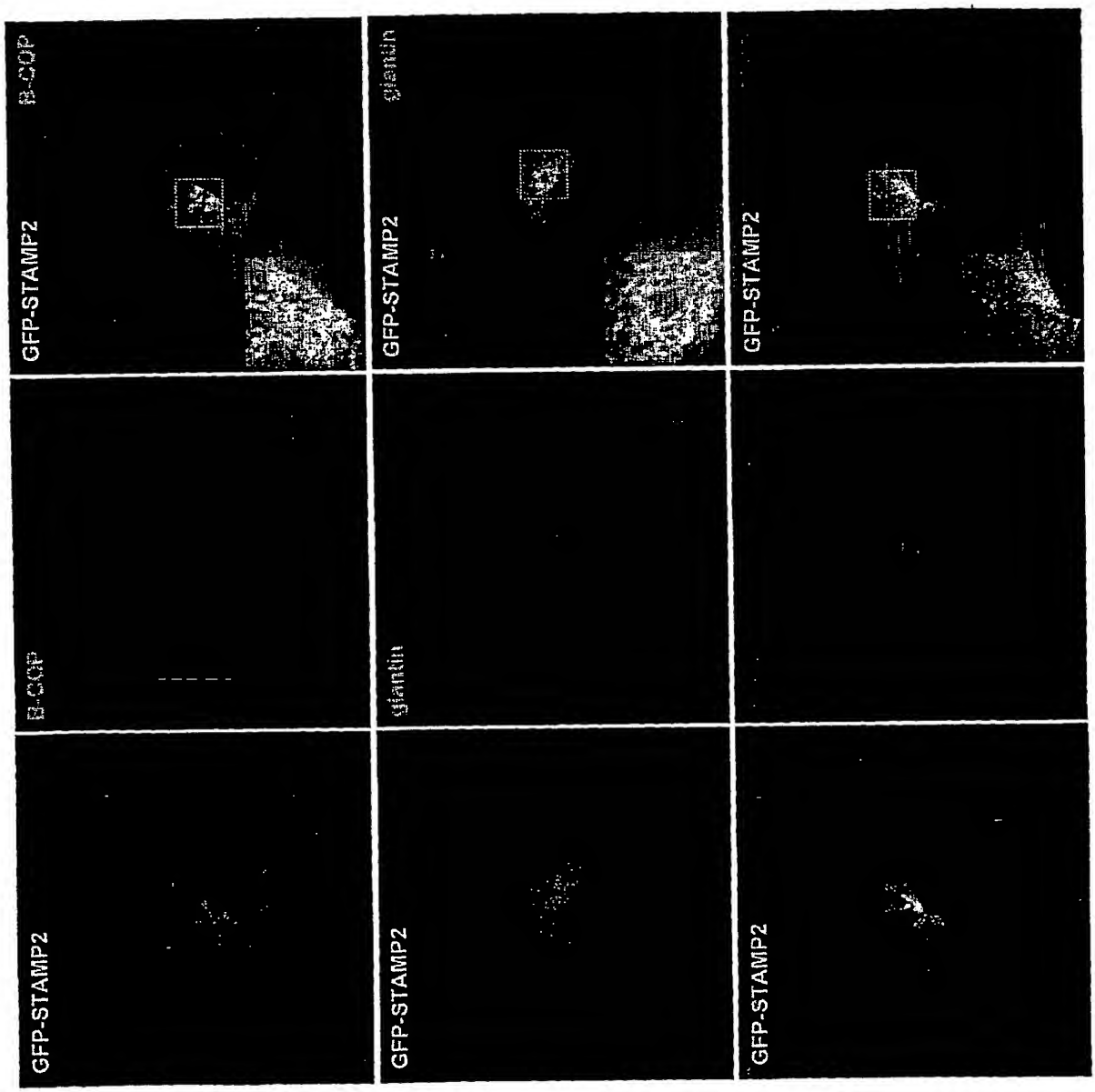
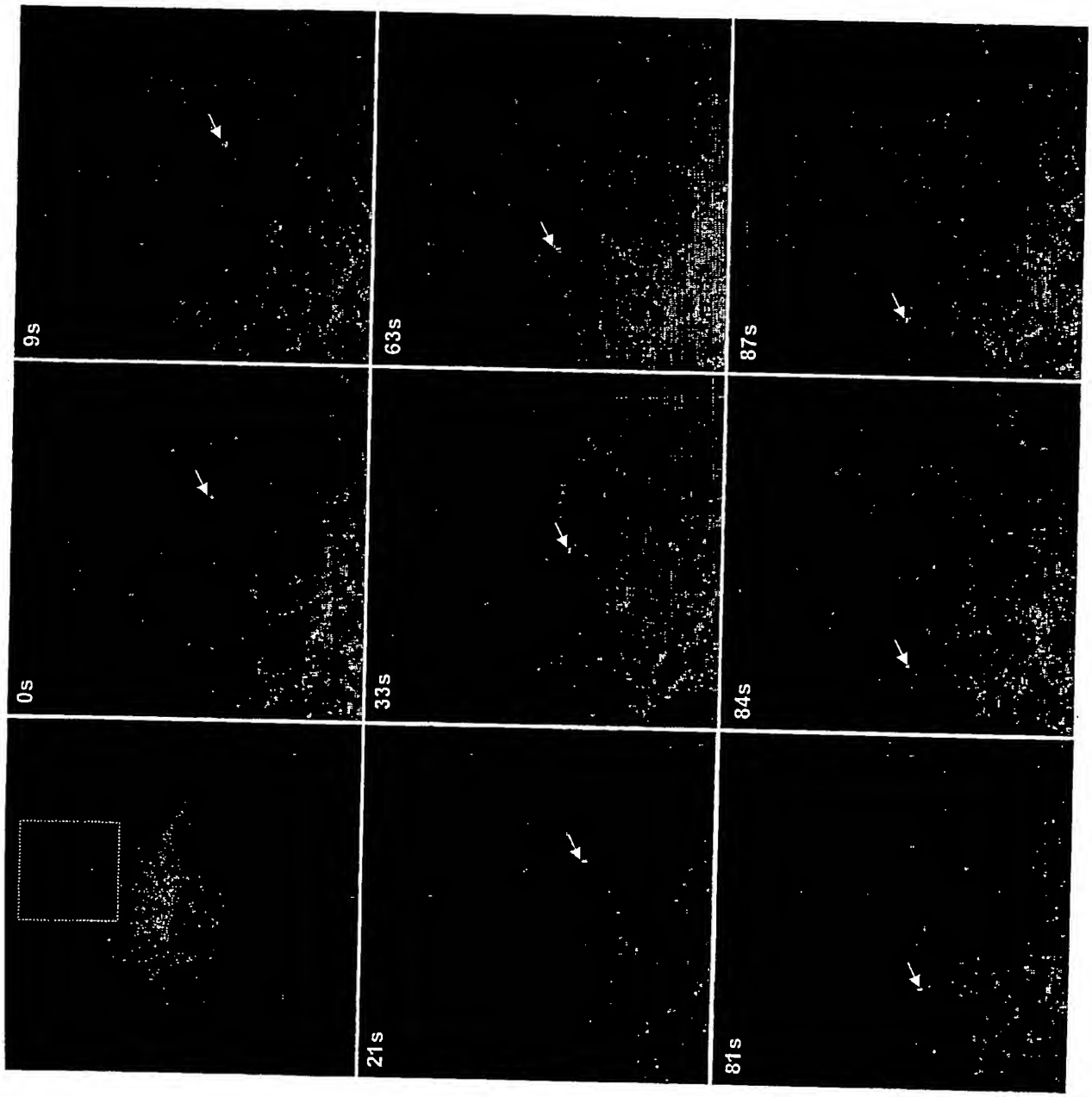

GFP-STAMP2
B-COP
GFP-STAMP2
B-COP
GFP-STAMP2
giantin
GFP-STAMP2
giantin
GFP-STAMP2
GFP-STAMP2

Figure 5B

Figure 6



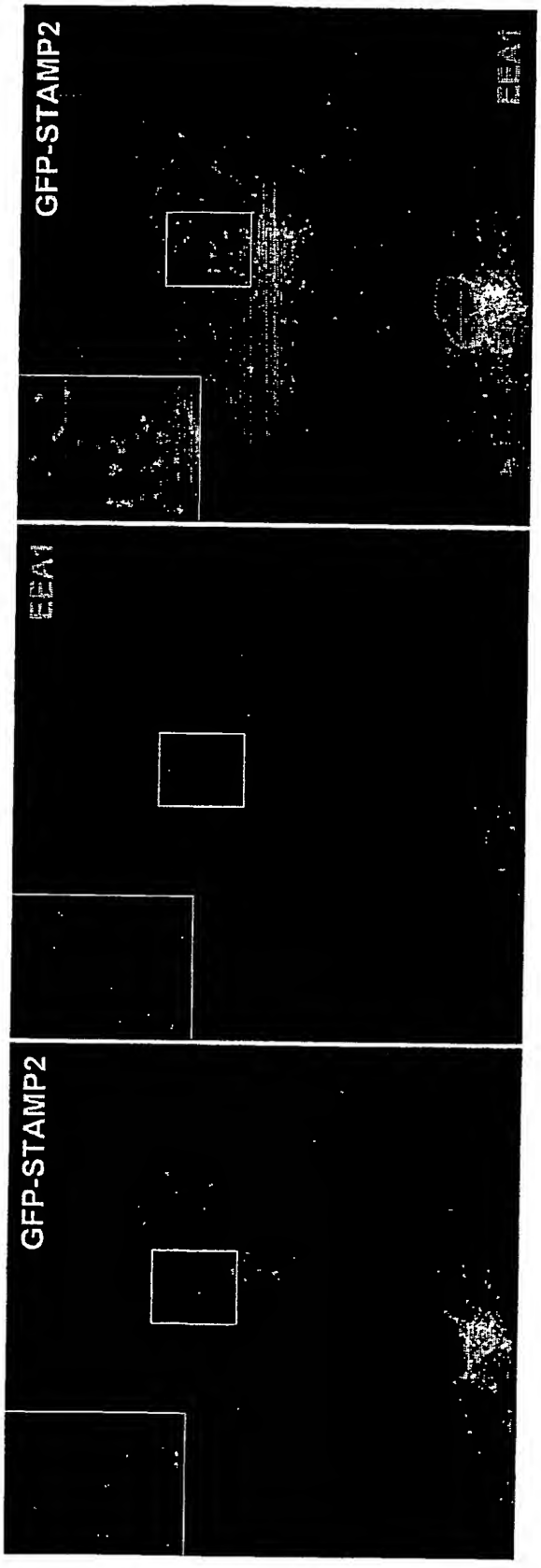
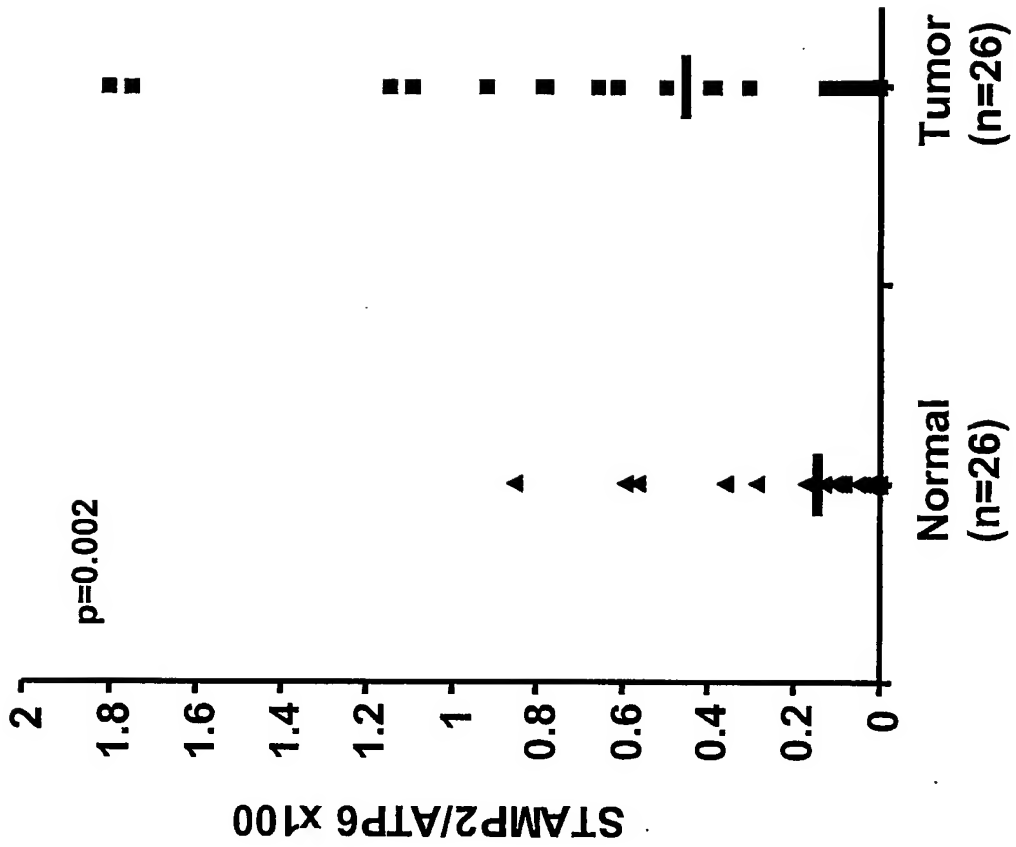


Figure 7

Figure 8


STAMP2/ATP6 x100
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
p=0.002
Normal (n=26)
Tumor (n=26)

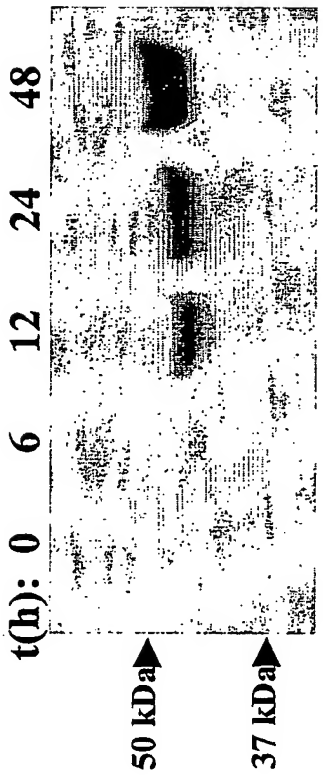


Figure 9

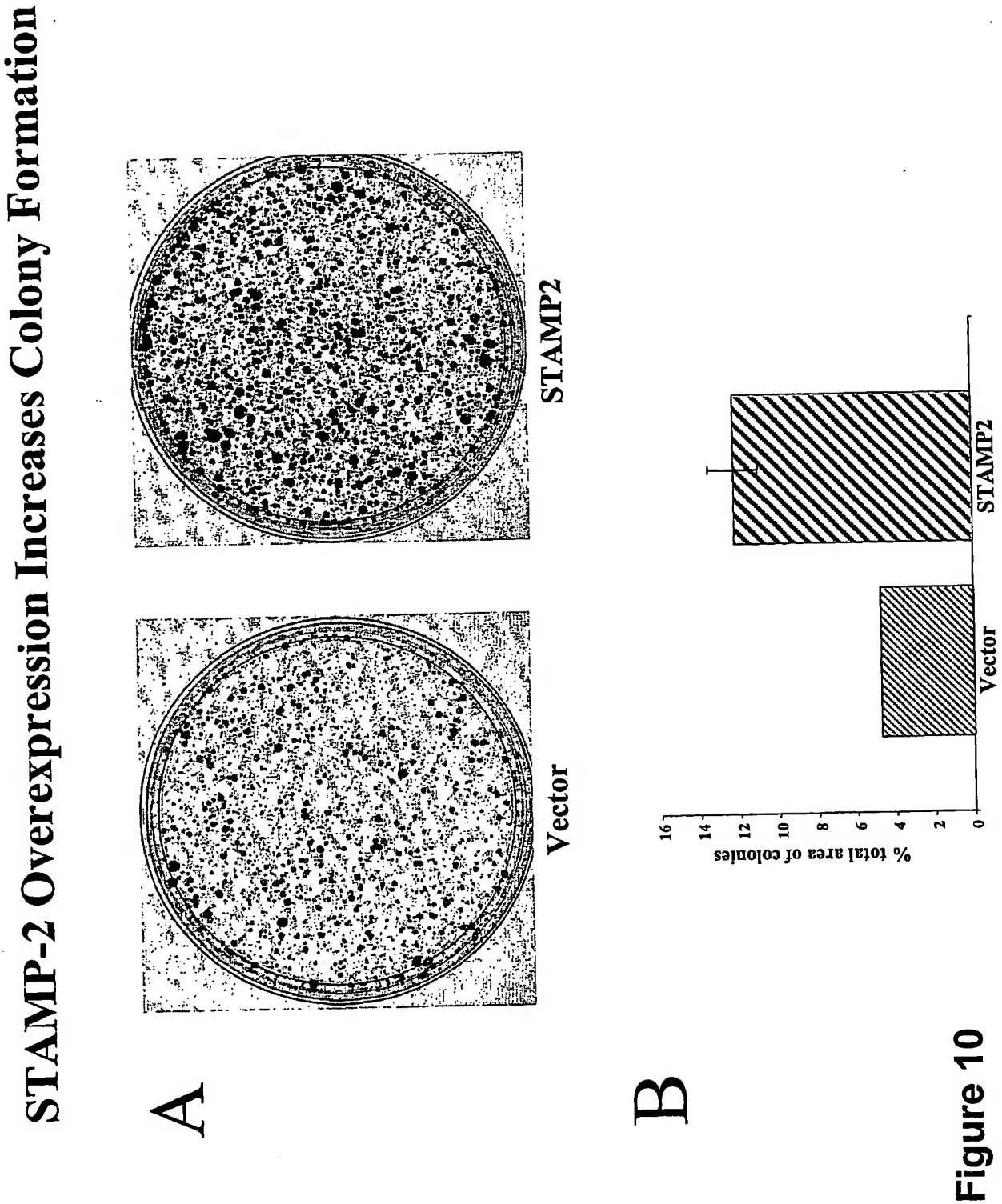

STAMP-2 Overexpression Increases Colony Formation
A
Vector
STAMP2
B
% total area of colonies
0
2
4
6
8
10
12
14
16
Vector
STAMP2

Figure 10

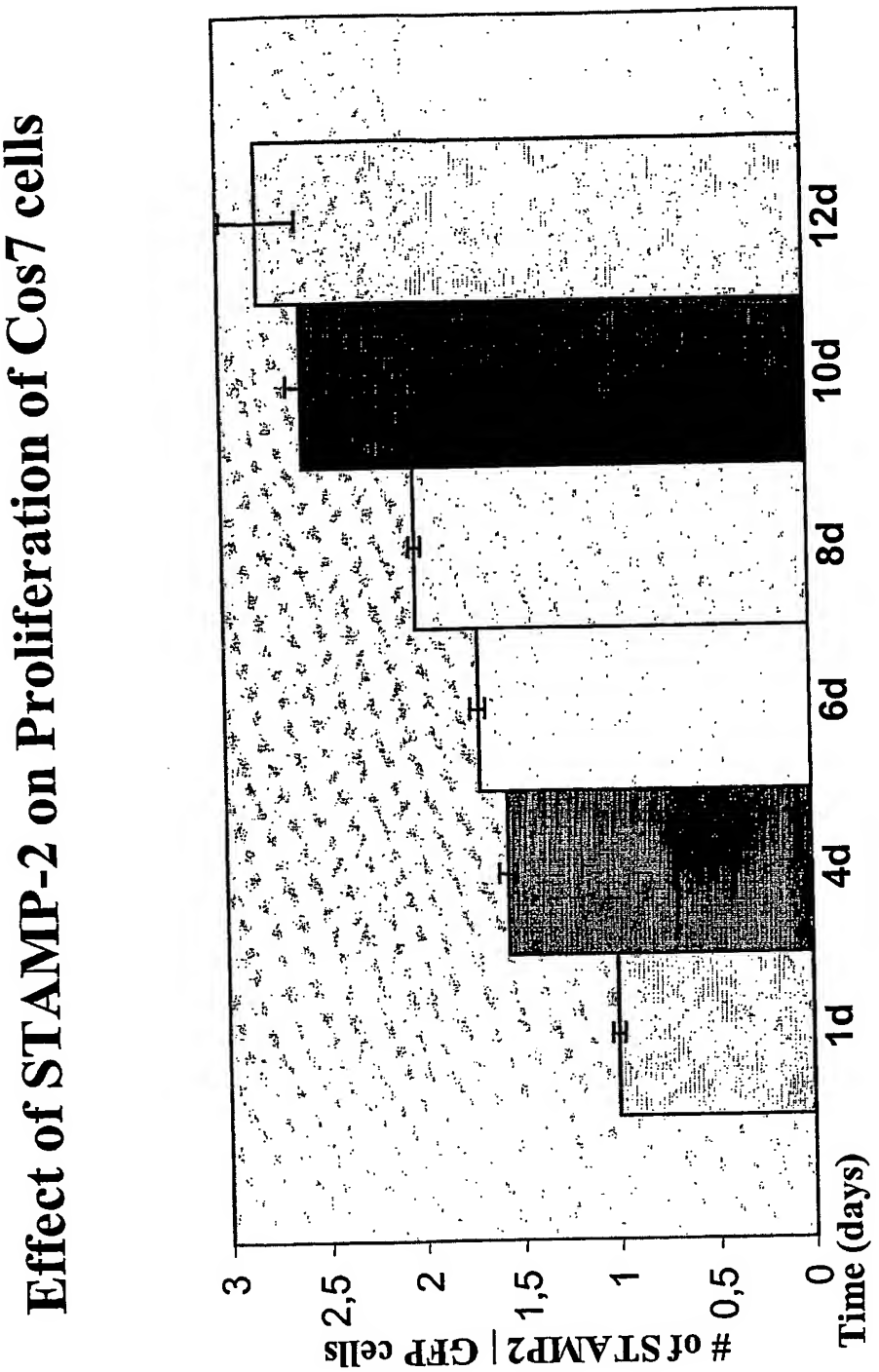

Effect of STAMP-2 on Proliferation of Cos7 cells
# of STAMP2 | GFP cells
3
2,5
2
1,5
1
0,5
0
Time (days)
1d
4d
6d
8d
10d
12d

Figure 11

Effect of STAMP-2 on Proliferation of DU145 cells

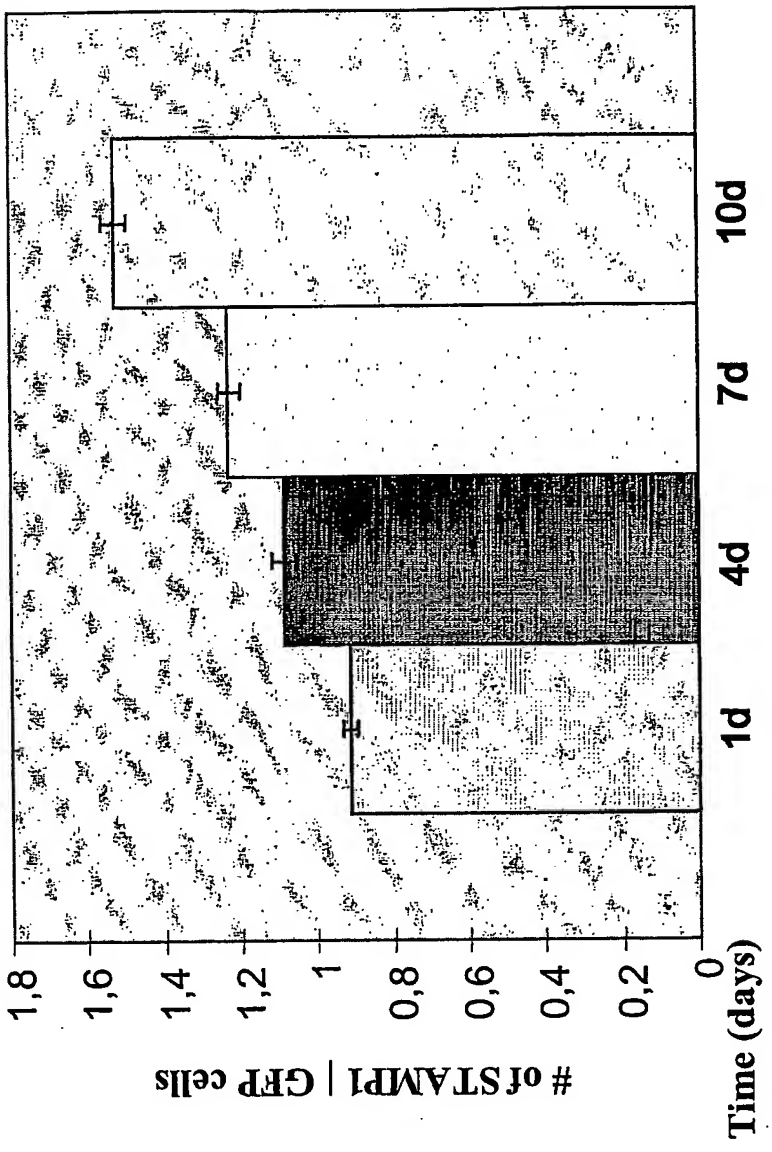


Figure 12